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Intrahippocampal cannabinoid administration induces antinociceptive and amnestic response

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ABSTRACT

In the present study, the possible role of a potent cannabinoid agonist, WIN55, 212-2 in the dorsal hippocampus on pain and memory performance has been evaluated. Animals were cannulated in CA1 region of the hippocampus using sterotaxic apparatus. Ten days after recovery, animals were trained in passive avoidance learning (PAL), and immediately received different doses of WIN 55212-2 (0.1, 0.25 and 0.5µg/rat), and were tested 24 h after the training. In the second part of the experiment animals received either WIN 55212-2 (0.5µg/rat) or saline respectively. Tail flick latency was measured three times with 10 minutes interval 30 minutes and 24 hours after the infusion into the CA1. Results indicate that post-training intra-CA1 administration of WIN55, 212-2 (0.25 and 0.5µg/rat) ($P< 0.001$) reduced step-down latency, showing an amnestic response. Also microinjection of WIN55212-2 (0.5ug/kg) into the CA1 region of the hippocampus induced analgesia compared to control group) ($P< 0.01$).

INTRODUCTION

The treatments of pain, nausea, seizures, ischemia, trauma and tumors are some of the potential therapeutic applications of cannabinoids (Russo, 2008; Carlini, 2004; Malan *et al.,* 2001). Cannabinoids (CBs) derived from the plant *Cannabis sativa,* and have been implicated in a variety of functions. It has been well documented that cannabinoids play a fundamental role in peripheral and spinal nociception (Palazzo *et al.,* 2010) For example in a rodent model of inflammatory pain, topical application of the CB, anandamide suppressed both development and maintenance of carrageenan-evoked thermal hyperalgesia (Richardson *et al.,* 1998; Nackley *et al.,* 2003). Also different models of neuropathic pain induced by nerve ligation have demonstrated a role for CB1 receptors in suppressing hyperalgesia and allodynia (Walker and Hohmann, 2005). However less attention has been paid on supraspinal effects. Studies revealed that Cannabinoid CB1 receptors are expressed in the brain and in certain peripheral tissues; CB2 receptors are mainly found in the immune system (Howlet *et al.,* 1990), but they have also been recently identified in the mammalian brain (Onaivi *et al.,* 2006).

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The presence of another receptor, CB3 has been documented too (Hayani and Davies 2000; Hajos *et al.,* 2001). Cannabinoids receptors are found in all of the nociceptive pathways including Para limbic area (Devane *et al.,* 1998; Dinh *et al.,* 2004). Experiment based on direct injections of CB agonists to specific brain regions such as PAG, dorsal raphe nucleus, RVM, amygdale, and thalamus have demonstrated the role of CBR in central nociception (Walker *et al.,* 1999). Interestingly the CB1 receptors are densely expressed in areas classically involved in learning and memory, such as the hippocampus and cortex (Davies *et al.,* 2002). The hippocampus is a component of the "limbic" system and it is assumed to contribute to the negative affect and avoidance motivation experienced during pain. According to some reports, hippocampus processes pain-related information, and some hippocampal neurons respond exclusively to painful stimulation, and that long-term anatomical changes occur in dentate gyrus neurons, following noxious physical stimulation (McKenna and Melzack 2001). Hippocampus not only has an established role in both pain and conditioned fear , but also it is a substrate for endocannabinoid activity (Ford *et al.,* 2011; Lisowski *et al.,* 2012). Regarding the processing mechanisms of sensory information in hippocampus, and existence of CB1 receptors, it is assumed that endocannabinoids in this area might contribute in both pain and learning functions.

Therefore the aim of this study was to investigate possible role of canabinoids in hippocampal processing of pain and inhibitory avoidance learning.

MATERIALS AND METHODS

Animals

In this study we used fifty male wistar rats weighing 200-250 g (16 for Tail flick, and 32 rats for PAL). They had free access to food and water, and kept at 24 ± 2◦C under a 12/12 h light dark cycle. All experiments were carried out on the morning. Each group consisted of eight animals and each animal was tested once. All experiments were conducted in accordance with the Guide for Care and Use of Laboratory Animals (National Institute of Health Publication No.80-23, revised 1996) approved by the Research and Ethics Committee of Guilan university.

Surgery

The animals were anesthetized intraperitoneally by a mixture of ketamine and xilazine (100 and 10 mg/kg, respectively). After being fixed in the stereotaxic apparatus (David Kopf Instruments, USA) with flat-skull position, the rat's scalp was cut, a small craniotomy was drilled and cannulas (22-gauge diameter) were bilaterally implanted into the CA1 region of hippocampus, at coordinates AP: −3 mm from bregma, L: ±2 mm from midline and V: −2.8 mm from the skull surface (Paxinos and Watson 2005).

Drugs and microinfusions

The drugs used in the present study were WIN55,212–2 mesylate (Tocris , UK). WIN55,212–2 was dissolved in vehicle (The vehicle were dimethylsulphoxide (DMSO; up to10% v/v) and sterile 0.9% saline and a drop of Tween 80). Control animals received saline and DMSO.

For bilateral drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulas and replaced by 27-gauge injection needles (1mm below the tip of the guide cannula). The injection solutions were administered in a total volume of 1 µl/rat (0.5 µl in each side) over a 60 s period. Injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs.

Behavioral procedures

Tail Flick

Nociception was assessed with tail-flick apparatus. One day before injection of the drugs, animals were exposed to the tail-flick apparatus to familiarize them with the procedure, in order to decrease the stress. Rats were wrapped in a towel and placed on the apparatus. The light source positioned below the tail, was focused on a point 2-3 cm rostral at the tip of the tail. Deflection of the tail activated a photocell and automatically terminated the trail. The tail-flick latency represented the period of time (sec) from the beginning of the trial to the tail deflection. Light intensity was adjusted so to obtain baseline tail-flick latencies of 3-4 s (0.7 mA). A cut-off time of 10 s was used to prevent tissue damage. In this part we used twenty rats which were divided into two groups of drug and vehicle. Tail flick latency was measured three times with 10 minutes interval, 30 minutes and 24 hours after infusion of the drug and vehicle.

Inhibitory avoidance apparatus

In this part we used thirty rats which were divided into three groups of different doses of WIN 55212-2 and one group of vehicle. The inhibitory avoidance apparatus consisted of a wooden box (40×30×40 cm high) with a steel-rod floor (0.3 cm in diameter, set 1 cm apart). A wooden platform (12×10× 7 cm) was set in the left side of the chamber. Intermittent electric shocks (100 Hz, 0.5 mA and 5 s) were delivered to the grid floor by an insulated stimulator. We used a single-trial step-down inhibitory avoidance task. Each rat was gently placed on the wooden platform. When the rats stepped down from the platform and placed all its paws on the grid floor, intermittent electric shocks were delivered continuously for 5s.

Retention test session was carried out 24 h after training and was procedurally identical to training, except that no shock was presented. Step- down latency was used as a measure of memory retention, an upper cut-off time of 300 s was set.

Histology

After the testing sessions each rat was deeply anesthetized and 1 ml of a 4% methylene-blue solution was bilaterally infused into the CA1 (0.5 ml/ side), then decapitated and its brain removed and placed in formaldehyde (10%). After several days, the brains were sliced and the sites of injections were verified according to Paxinos & Watson, 2005 (Figure1). Data from the animals with the injection sites located outside the CA1 (less than %5) were not used in the analysis.

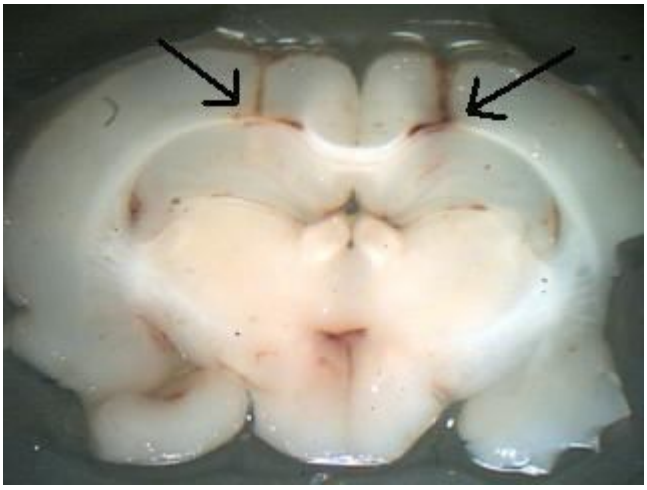

Fig.1. A rat brain section showing the extension of the area reached by infusions into the hippocampus in the animals with correct infusion placements.

Data analysis

Data obtained from tail flick were analyzed using K-Smirnov and after approving the normality of data, student t-test

was used. The data are expressed as mean ± S.E.M. Data obtained from passive avoidance were analyzed with one way analysis of variance (ANOVA). Post-hoc comparison of means was carried out with the Tukey test for multiple comparisons, when appropriate. The level of statistical significance was set at $P<0.05$. Calculations were performed using the SPSS statistical package ver 19.

RESULTS

Microinjection of WIN 55212-2 (0.5µg) into the CA1 induces analgesia compared to control group ($P< 0.01$). The analgesic effect of the drug lasted for at least 24 hours (Fig. 2). In the second part of the experiment, the effects of post-training administration of WIN55, 212-2 on memory of inhibitory avoidance task were examined. Five groups of animals received vehicle (1µl/rat, intra-CA1) or the different doses of WIN55, 212-2 (0.1, 0.25 and 0.5 µg/rat, intra-CA1) immediately after the training.

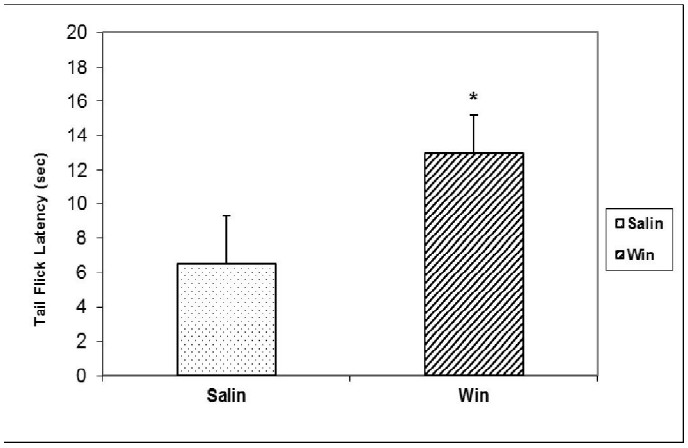

Fig. 2. The effects of WIN55,212-2 on tail flick latency. Two groups of animals received vehicle (1µl/rat), or 0.5 µg/rat WIN55,212-2. Test session tail flick latencies are expressed as mean for eight animals.*p < 0. 05 different from the vehicle group. There were 8 animals in each group.

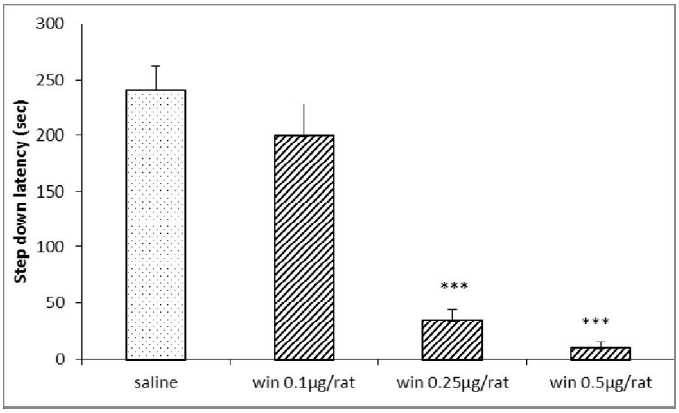

Fig. 3 The effects of WIN55,212-2 on memory retention. Four groups of animals received post-training vehicle (1µl/rat), or different doses of WIN55,212-2 (0.1, 0.25 and 0.5 µg/rat). Test session step-down latencies are expressed as median and quartile for eight animals. ***p<0.001 different from the vehicle group., There were 8 animals in each group.

Fig.3 shows the effects of post- training intra-CA1 administration of WIN55, 212-2 on step-down latency. One-way ANOVA revealed that post-training WIN55, 212-2 (0.25 and 0.5 µg/rat) reduced the step-down latency in the inhibitory avoidance task [$F (3,33)= 26.39$, $P<0.001$], showing that intra-CA1 administration of WIN55, 212-2 induced amnesia.

DISCUSSION

In the present study we used Tail flick method which measures phasic pain. The result of this study showed that intrahippocampal infusion of CB1 receptor induces analgesic effect in a superficial pain test. Similar finding for the involvement of supraspinal cannabinoid receptors in the modulation of pain has been reported from intra cerebral microinjection of cannabinoids in animal models of acute, inflammatory or neuropathic pain (Walker *et al.,* 1999; Davies *et al.,* 2002; Lisowski *et al.,*2012). For example analgesic effect of cannabinoids in chronic pain induced by formalin or capsaicin administration has been documented in the studies carried out by De Novellis and Sanjay (De Novellis *et al.,* 2005; Sanjay *et al.,* 2004). Our result indicates that nociceptive information is processed by the hippocampus, and at least the cannabinoid signaling in the CA1 plays an important role in nociceptive functions. Analgesic effect induced by CB1 agonist in our study confirm the involvement of CB1R through activation of G protein -coupled CB1Rs which was represented in previous studies (Pertwee 2001; Hohmann and Herkenham 1999).

On the other hand the results of second part of our experiments indicated that post-training intra-CA1 administration of non-selective cannabinoid receptor agonist, WIN55,212-2 impaired memory retrieval on the test day. Since WIN55,212-2 was infused immediately after the training, the amnestic effect observed here, reflects that cannabinoid CB1 receptor agonist effects on the process of memory acquisition and/or consolidation. Our finding is in agreement with other behavioral data obtained from different methodologies for learning and memory paradigm (Kobilo *et al.,* 007; Robinson *et al.,* 2008; Goonawardena *et al.,* 2010a; Goonawardena *et al.,* 2010a ; Robinson *et al.,* 2007 ; Seillier *et al.,* 2010). For example ssystemic administration of a plant-derived cannabinoid receptor agonists such as Δ9-Tetrahydrocannabinol (Δ9-THC), WIN55,212-2 and HU-210 impair working memory and short-term memory in rodents (Robinson *et al.,* 2008; Goonawardena *et al.,* 2010a; Goonawardena *et al.,* 2010a ; Robinson *et al.,* 2007). Also our finding is in line with evidences that SR141716A , a well-known CB1 receptor antagonist impairs extinction learning in the Morris water maze and extinction of conditioned freezing (Marsicano *et al.,* 2002; Suzuki *et al.,* 2004) suggesting that an endocannabinoid tone is crucial for various forms of learning and memory. High levels of expression of CB1 receptors in the hippocampus support the effectiveness of WIN55, 212-2 on pain and memory formation (Herkenhham *et al.,* 1990; Pettit *et al.,* 1998).

The exact mechanisms of cannabinoids in modulation of learning and memory have not been well understood. There is evidence indicating that cannabinoids involve in neurotransmitters

transmission in the brain (Schlicker and Kathmann 2001). Generally cannabinoids are able to inhibit glutamate release (Shen *et al.,* 1996) acetylcholine release (Gifford *et al.,* 1997) GABA (Hofmann *et al.,* 2011) and noradrenaline (Gifford *et al.,* 1997) in cultured rat hippocampal cells. Although CB1R is found in both GABAergic and glutamatergic neurons in the hippocampal CA1 region (Hofmann *et al.,* 2011) is more abundant in GABAergic interneurons than in glutamatergic principal neurons (Al-Hayani and Davies 2002; Marsicano and Lutz 2006). Therefore it is more likely that cannabinoids induce the release of GABA rather than inhibit presynaptic excitatory neurotransmitters release (Kawamura *et al.,* 2006). However, one cannot exclude the finding that Cannabinoids reduce hippocampal long-term potentiation (Bellocchio *et al.,* 2010).

Taken together, our finding suggest that administration of WIN55,212-2, a cannabinoid receptor agonist, into the CA1 increases the thermal latency to withdrawal in the tail-flick test and reduce step-down latency. Therefore, cannabinoid system could play a pivotal role in modulating memory and pain processes.

CONCLUSIONS

Our findings indicates that CBs-dependent analgesia occur in supra spinal region and activation of cannabinoid receptors might be of clinical significance in management of pain. However limitations to therapeutic approaches which modulate the endocannabinoid system should be considered due to amnestic effect of these drugs.

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