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Cannabinoid receptor agonism suppresses tremor, cognition disturbances and anxiety-like behaviors in a rat model of essential tremor

Hassan Abbassian [a], Parisa Esmaeili [b], Mahshid Tahamtan [a], Iraj Aghaei [c,d], Zohreh Vaziri [a], Vahid Sheibani [a], Benjamin J. Whalley [e,*], Mohammad Shabani [a,*]

[a] Neuroscience Research Center, Neuropharmacology Institute, Kerman University of Medical Sciences, Kerman, 76198-13159, Iran
[b] Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[c] Cellular and Molecular Research Center, Guilan University of Medical Sciences, Rasht, Iran
[d] Department of Nursing, Guilan University of Medical Sciences, Rasht, Iran
[e] Department of Pharmacy, School of Chemistry, Food & Nutritional Sciences and Pharmacy, University of Reading, Whiteknights, Reading, Berkshire, RG6 6AP, UK

HIGHLIGHTS

- CBR agonist typically ameliorated harmaline induced tremor.
- WIN affected explorative and gait disturbances induced by harmaline.
- CBR agonist improved impairments of anxiety-like behaviors following harmaline.
- WIN reversed balance and passive avoidance learning impairment induced by harmaline.

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ABSTRACT

Cognitive and motor disturbances are serious consequences of tremor induced by motor disorders. Despite a lack of effective clinical treatment, some potential therapeutic agents have been used to alleviate the cognitive symptoms in the animal models of tremor. In the current study, the effects of WIN55, 212-2 (WIN), a cannabinoid receptor (CBR) agonist, on harmaline-induced motor and cognitive impairments were studied. Adult rats were treated with WIN (0.5 mg/kg; i.p.) 15 min before harmaline administration (10 mg/kg; ip) after which exploratory and anxiety related behaviors, and cognitive function were assessed using open-field behavior and shuttle box tests. Rats that received harmaline only exhibited a markedly reduced number of central square entries when compared to harmaline vehicle-treated controls, whereas those treated with WIN and harmaline showed a significant increase in central square entries, compared to harmaline only treated. The passive avoidance memory impairments observed in harmaline treated rats, was reversed somewhat by administration of WIN. The neuroprotective and anxiolytic effects of WIN demonstrated in the current study can be offered cannabinoid receptor (CBR) agonism as a potential neuroprotective agent in the treatment of patients with tremor that manifest mental dysfunctions.

1. Introduction

Essential tremor (ET) is conventionally conceived of as a purely motor disease and some studies have revealed an association between ET and increased risk for cognitive impairment and dementia, which suggests that cognitive impairments in ET patients may be a consequence of an additional neurodegenerative disorder. However, other studies have identified cognitive deficits in ET patients as being frontosubcortical or corticocerebellar which are consistent with symptoms arising in whole or in part from ET itself and independent of medication used to treat ET symptoms [18]. Furthermore, no pharmacotherapies to treat cognitive deficits in ET patients have been developed, revealing an unmet clinical need in this population.

Predictive animal models of symptoms and disease remain an important element of drug development. Systemic harmaline administration causes action tremor in mammals and has proved to be a useful

Abbreviations: WIN, WIN 55,212-2; CBR, cannabinoid receptor; ET, essential tremor; ACPA, arachidonyl-cyclo-propyl-amide.

* Corresponding authors.
E-mail addresses: b.j.whalley@reading.ac.uk (B.J. Whalley), shabanimoh@yahoo.com, shabani@kmu.ac.ir (M. Shabani).

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animal model for the discovery of new therapies for primary symptoms of ET [8]. Furthermore, in addition to harmaline causing agitation, cytotoxicity, delirium, paralysis, loss of coordination, tremor, visual disturbances and hallucinations [20], it has also been reported to induce cognitive disturbances, most likely as sequelae to low harmaline doses (5–10 mg/kg) acting anxiogenically or higher doses (20 mg/kg) exerting reportedly anxiolytic effects in rodents [15]. It has also reportedly affected emotional reactivity in mice as decision making in an anxiogenic situation can be altered by harmaline treatment [15] in addition to inducing cognitive disturbances that manifest as motor and spatial learning impairments [15]. Therefore, the symptoms exhibited by rodents following systemic harmaline administration are consistent with being predictive for drug effects upon cognitive domains of interest to human ET pharmacotherapy.

The endocannabinoid system is implicated in cognition and genetic deletion of cannabinoid type 1 (CB_1) receptors accelerates age-related cognitive decline in rodents [19], accompanied by neuronal loss in the CA1 and CA3 regions of the hippocampus [6]. CB_1 receptors are presynaptically located where activation reduces presynaptic neuronal excitability and so inhibits neurotransmitter release [16]. CB_1 receptor expression is abundant in several brain regions including the hippocampus, prefrontal cortex, nucleus accumbens and amygdala where their modulation of neurotransmitter release exerts a variety of behavioral and cognitive effects [20]. Here, a substantial body of evidence from animal models and human studies has shown that CB_1 receptor agonists, frequently in the form of Δ^9-tetrahydrocannabinol which is the principal psychoactive component derived from *Cannabis sativa*, induce numerous and complex effects on cognitive functions including attention, learning, emotional reactivity, enhancement of the perceptions of the senses, and, idiosyncratically, impairment and improvement in short-term memory [5,30,34,35]. In the passive avoidance task, CB_1 receptor activation reversed opioid-induced memory impairment [41] but in other reports have been shown to impair passive avoidance learning in addition to adversely affecting spatial and working memory [14,33]. For example, the CB_1 receptor agonist, arachidonylcyclopropylamide (ACPA) induced memory acquisition impairment in mice which was reversed by co-administration of a CB_1 receptor antagonist [26]. Interestingly, prenatal administration of the CB receptor agonist, WIN55, 212-2 (0.5–1 mg/kg) during embryonic days 5–20 can disrupt memory retention in offspring when assessed at P30–P35 using the passive avoidance task [33]. A role for CB_1 receptors in memory consolidation was shown by treatment with the CB_1 receptor selective antagonist, rimonabant (0.1 mg/kg and 1.0 mg/kg; i.p.), which caused significant improvement in passive avoidance performance [2]. Moreover, alterations in the sleep–wake cycle, memory formation, locomotor activity and pain perception have been widely reported in studies of the effects of the endocannabinoid, anandamide [4,11].

In the present study, we examine the effect of harmaline at a reportedly low, anxiogenic dose (10 mg/kg; i.p.) upon tremor, gait, anxiety and associative learning and memory in rats before investigating the effects of cannabinoid receptor agonism upon harmaline-induced effects in these domains. Here, harmaline produced a moderate and persistent tremor, gait disturbances, increased anxiety and a significant impairment in the learning and recall capability in the passive avoidance task. While prior CBR agonist treatment had no effect upon harmaline-induced tremor or gait disturbances, the anxiogenic effects of harmaline were attenuated and some impairments of memory formation and retention were reversed.

2. Methods and materials

2.1. Animals

30 adult, male Wistar Kyoto rats (60–80 g) were used. Animals were kept in individual cages with access to food and water *ad libitum* and maintained on a 12 h/12 h dark/light cycle. Every effort was made to minimize animal suffering during all stages on the study. All procedures were approved by the Kerman Medical University Ethics Committee (EC/KNRC/92-63).

2.2. Drugs

The non-selective cannabinoid type 1 (CB_1) and type 2 (CB_2) receptor agonist, WIN55, 212-2 (WIN; Sigma, USA), was dissolved in dimethylsulfoxide (DMSO) before 100-fold dilution in normal saline. Harmaline hydrochloride dihydrate (Sigma) was dissolved in normal saline.

2.3. Behavioral tasks

2.3.1. Tremor scoring

Tremor was rated by two observers blinded to treatment. Intra- and inter-observer reliability were assessed *via* kappa coefficient (acceptance criterion: >80%). Tremor data were acquired during the open field test and quantitatively scored as follows: 0: No tremor, 1: occasional tremor affecting only the head and neck, 2: intermittent (occasional tremor affecting all body parts), 3: persistent (persistent tremor affecting all body parts and tail), 4: severe (persistent tremor rendering the animal unable to stand and/or walk) [3].

2.3.2. Gait analysis

The footprint test assesses animal walking patterns and gait kinematics. The hind paws of each animal were marked with a non-toxic ink and the animal allowed to traverse a clear Plexiglas tunnel (100 cm [L] × 10 cm [H] × 10 cm [W]) lined with white absorbent paper (100 cm × 10 cm) and ending in a darkened cage. The resulting tracks provide the spatial relationship of consecutive footfalls from which animal stride length and width were measured. Animals were habituated to the runway for 3 training runs before testing. Hind paw stride lengths were measured by distance (cm) between the respective paw prints to the successive ipsilateral prints to assess uni- or bi-lateral effects of treatment upon gait. Hind paw stride widths were measured by distance between the centers of the respective paw prints to the corresponding contralateral stride length measurements at a right angle. Footprints at the beginning and end of each run were not considered in the analysis [40].

2.3.3. Open-field test

The open field apparatus consisted of a square Plexiglas arena (90 [W] × 90 [L] × 45 [H] cm), the floor of which was divided by lines into 16 squares to define central and peripheral regions. Each animal in turn was placed in the middle of the open field apparatus and vertical (rearing) and horizontal activity video recorded for a five-minute period. Video recordings were analyzed offline using EthoVision (Noldus Information Technology, Netherlands) video tracking software for automated classification of behavioral paradigms and the following parameters recorded for each animal: total distance moved (cm) and time spent in peripheral and central regions (seconds). At the end of each test, the animal was removed from the chamber and the field cleaned with 70% ethanol [29].

2.3.4. Passive avoidance test

The passive avoidance task is a fear-aggravated test used to evaluate associative learning and memory in rodents. The animal learns to avoid an environment in which a prior aversive stimulus has been delivered. Here, passive avoidance learning was assessed using an inhibitory passive avoidance paradigm as described hereafter. Briefly, a shuttle-box device with dimensions of 100 [L] × 25 [W] × 25 [H] (cm) and consisting of two compartments (light and dark) separated by a door was used. In the learning phase of the test, each animal was first habituated to the test equipment by placement in the light chamber (door closed) for