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Interaction between cannabinoid compounds and diazepam on anxiety-like behaviour of mice

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Abstract

Previous studies have suggested that cannabinoidergic system is involved in anxiety. However, a complete picture of cannabinoid association in the anxiety is still lacking. In the present study, we investigated the possible interaction between cannabinoidergic and GABAergic systems in the anxiety-like behaviour of mice. Intraperitoneal (i.p.) administration of the cannabinoid receptor agonist WIN55212-2 (0.25–5 mg/kg), the endocannabinoid transport inhibitor AM404 (0.25–2 mg/kg) and diazepam (0.25–8 mg/kg) dose dependently exhibited an anxiolytic effect evaluated in terms of increase in the percentage of time spent in the open arms in the elevated plus maze (EPM) test. Administration of certain fixed-ratio combinations (3:1 and 1:1) of WIN55212-2 and diazepam produced a synergistic anxiolytic effect, while the 1:3 combination produced an additive effect. In hole-board test, administration of certain ratios of WIN55212-2–diazepam combination significantly altered the animal behaviour compared to groups that received each drug alone. Co-administration of AM404 (1 and 2 mg/kg) and diazepam (0.5 mg/kg) abolished the anxiolytic effect of the former drug in EPM and the latter in hole-board test, respectively. The combination of an ineffective dose of the fatty acid amide hydrolase (FAAH) inhibitor, URB597 (0.3 mg/kg, i.p.) on anxiety-related responses with an ineffective dose of diazepam (0.25 mg/kg, i.p.) led to a synergistic effect. Co-administration of the CB1 receptor antagonist, AM251 (5 mg/kg) and an effective dose of diazepam (2 mg/kg, i.p.) attenuated diazepam-induced elevation of percentage of time spent in open arm, while lower dose of AM251 (0.5 mg/kg) failed to inhibit diazepam-induced anxiolytic effect. Taken together, the present study showed that co-administration of exogenous cannabinoids and diazepam produce additive or synergistic effect at different combinations. Moreover, it has been shown that enhancement of the function of endocannabinoids could increase the anxiolytic effect of diazepam.

Keywords: Anxiety; Cannabinoids; Diazepam; GABA; Elevated plus maze; Hole-board; Mice

1. Introduction

Several findings suggest that cannabinoid system, through the activation of cannabinoid CB1 receptors, is involved in the modulation of anxiety-related behaviour (Piomelli et al., 1998; Viveros et al., 2005; Patel and Hillard, 2006). However, the anxiety-related effects of cannabinoids remain controversial as agonists show opposite effects in different studies. Some authors suggested that the anxiolytic or anxiogenic action of cannabinoid CB1 agonists is dose-dependent. For instance, it has been shown that CP55940 and WIN55212-2, two potent cannabinoid receptor agonists, produce anxiolytic effects in mice submitted to the elevated plus maze (EPM) model of anxiety, at low doses only (Patel and Hillard, 2006). However, higher doses of these compounds could produce an anxiogenic profile (Viveros et al., 2005). Another hypothesis suggested that cannabinoid action on anxiety-like behaviour of animals is species-dependent. Based on behavioural and electrophysiological findings, Haller et al. (2007a,b) showed that WIN5521-2 reduced anxiety in mice by affecting GABA neurotransmission, whereas it increased anxiety in rats via glutamatergic mechanisms. The results of several

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studies are consistent with Haller's hypothesis. For instance, the natural active ingredient of cannabis plant, delta-9-tetrahydrocannabinol (THC) produced the anxiolytic effects on mice in the light–dark test (Berrendero and Maldonado, 2002). In contrast, THC was anxiogenic in adult rats submitted to the EPM and light–dark tasks (Schramm-Sapyta et al., 2007). Regarding the role of endocannabinoids on anxiety, the results are still controversial. Several studies suggest an anxiolytic role for these compounds. For instance, anxiety is increased by both the genetic disruption of the CB1 receptor and its pharmacological blockade by AM-251 or rimonabant in mice (Navarro et al., 1997; Haller et al., 2002, a,b; Uriguen et al., 2004; Patel and Hillard, 2006). It has been shown that stress may accompany the reduction in endocannabinoid levels at synapses, suggesting a tonical release of endocannabinoids under resting condition in some regions of the brain which are involved in coping with stress (Patel et al., 2004). In contrast, conflicting data has been reported using cannabinoid CB1 receptor mutant mice in the shock-probe burying test (Degroot and Nomikos, 2004). In rats, the anxiolytic effect of endocannabinoids seems to be more dominant. Pharmacological blockade of the enzyme fatty acid amide hydrolase, which is responsible for intracellular anandamide degradation, produces anxiolytic effects on adult rats tested in the elevated zero maze (Gaetani et al., 2003) and in the isolation-induced ultrasonic vocalization paradigm in rat pups (Kathuria et al., 2003). The peripheral injection of the anandamide transport inhibitor, AM404, exhibited anxiolytic-like effects in different rat models of anxiety. These effects were accompanied by an increased brain level of anandamide and were prevented by cannabinoid CB1 receptor blockade (Bortolato et al., 2006; Rutkowska et al., 2006).

Anatomical studies have shown that CB1 receptors are widely distributed in the brain structures involved in emotional control including basolateral amygdala, cortical (the entorhinal, cingulate, frontal and prefrontal) regions and the hippocampus (Breivogel and Childers, 1998; Herkenham et al., 1990). As a result of this localization, CB1 activation might have a complex pattern of influence upon neurotransmitters known to modulate anxiety (Arevalo et al., 2001; Martin et al., 2002; van der Stelt and Di Marzo, 2003). In addition, cannabinoids could activate the hypothalamic pituitary–adrenal axis which is responsible for the neuroendocrine response to stress (Weidenfeld et al., 1994). However, the exact mechanism by which cannabinoids modulate anxiety-related behaviour is not elucidated yet.

The GABAergic system, in particular $GABA_A$, has a pivotal role in the regulation of anxiety and benzodiazepines are still the most widely used anxiolytic compounds (Roy-Byrne, 2005). Electrophysiological studies have shown that endogenous cannabinoids (eCBs) can retrogradely suppress inhibitory neurotransmitter release at synapses. This type of modulation has been shown in different regions of the brain including structures involved in emotional control such as amygdala (Zhu and Lovinger 2005), prefrontal cortex (Melis et al., 2004) and hippocampus (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). Involvement of GABAergic neurons in mediating cannabinoid effects on feeding behaviour has been already reported (Rahminiwati and Nishimura, 1999). However, little attention has been paid to the interaction between cannabinoid and GABAergic system to control anxiety-like behaviour. On the basis of the above evidence, the present study was designed to investigate the interaction between cannabinoidergic and GABAergic systems on anxiety-like behaviour in two models of anxiety in mice: the elevated plus maze (EPM) and hole-board test. In order to test this hypothesis, we utilized cannabinoid compounds anandamide transport inhibitor (AM 404), the cannabinoid receptor agonist (WIN55212-2), the fatty acid amide hydrolase (FAAH) inhibitor (URB597) and the cannabinoid receptor antagonist (AM251).

2. Materials and methods

2.1. Animals

The experiments were carried out on male NMRI mice (Pasteur Institute, Karaj, Iran) weighting 20–25 g. The animals were maintained at 22 °C on a 12 h light–dark cycle with food and tap water available *ad libitum*. All procedures were in accordance with the Shaheed Beheshti University of Medical Sciences Guideline for the Care and Use of Laboratory Animals and were approved by the local Research and Medical Ethics Committee.

2.2. Drugs

Diazepam, WIN55212-2, AM404 and AM251 were obtained from Sigma-Aldrich, Steinheim, Germany. The drugs except for URB597 were suspended in vehicle (80% saline, 18% dimethylsulfoxide (DMSO), 1% emulphor, 1% ethanol) and were delivered by intraperitoneal (i.p.) injection at a volume of 10 ml/kg. URB597 was dissolved in DMSO and was delivered by i.p. injection at a constant volume of 50 μl.

2.3. Elevated plus maze test

Activity and anxiety-related behaviours were assessed using the mouse elevated plus maze (EPM) test (Dawson and Tricklebank, 1995; Lister, 1987; Pellow et al., 1985). The apparatus consists of two open and two enclosed horizontal perpendicular arms (30 × 5 cm) positioned 40 cm above the floor. The junction of four arms forms a central square platform (5 × 5 cm). All drugs, either individually or in combination, were given 30 min before submitting the animal to the EPM apparatus. Each animal was placed in the central platform facing one of the open arms and allowed to explore freely for 5 min. Between each trial, the maze was thoroughly cleaned with 10% ethanol solution and afterwards by a dry cloth. The experiments were conducted under artificial laboratory illumination (fluorescent lamps, 80 lx at maze level). The sessions were recorded by a camera positioned right above the maze hanging from the ceiling. Data were obtained using Ethovision software (version 3.1), a video tracking system for automation of behavioural experiments (Noldus Information Technology, the Netherlands). During the 5 min trial, the behaviour of each mouse was recorded as: (i) the number of entries into the open or closed arms and (ii) average time spent by mouse in each of the arms. The number of entries into open arms