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Research report

Behavioral evidence for the interaction between cannabinoids and *Catha edulis* F. (Khat) in mice

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ABSTRACT

Several studies have shown the existence of an interaction between the endocannabinoid system and some drugs of abuse, such as opioids, nicotine, alcohol, and cocaine. For instance, the endocannabinoid system has long been known to play a role in the underlying mechanisms of drug reward and dependence. The aim of this study was to evaluate the possible existence of an interaction between the endocannabinoid system and khat after acute administration. Behavioral interactions of khat extract with cannabinoids were assessed. To this effect, mice were randomly divided into different groups (vehicle, khat extract, khat and WIN55,212-2, a cannabinoid agonist, khat extract and cannabinoid antagonists, AM251 & AM630) and their behavioral responses were evaluated in activity monitor, elevated plus maze and Y-maze tests. These tests were used to determine changes in locomotor activity, anxiety-like behavior, and working memory. Khat and WIN55,212-2 demonstrated differential responses in these tests, but co-administration of these agents invariably increased the measured parameters, which were reversed by the cannabinoid receptor antagonists used. The data collectively indicate that there is an interaction between khat and the endocannabinoid system, which most likely involves the cannabinoid receptors or a common mechanism separately activated by the two agents.

1. Introduction

Cannabinoids constitute a diverse class of compounds that include Δ^9-Tetrahydrocannabinol (Δ^9-THC), the principal psychoactive constituent of marijuana, and synthetic compounds such as HU-210. Administration of cannabinoids in animal models produces various behavioral changes, mediated by cannabinoid receptors (CBRs) (Ledent et al., 1999). Elements of the endocannabinoid system include the two subtypes of CBRs (CB_1R and CB_2R) and their endogenous ligands; the endocannabinoids (eCBs) like anandamide, a partial agonist, and 2-arachidonoyl glycerol(2-AG), a full agonist at the CBRs; along with the enzymes involved in their synthesis and degradation (Onaivi et al., 2012). The CB_1Rs can be found in several areas of the brain such as the frontal cortex, basal ganglia, hippocampus, amygdala and brainstem and they have been implicated in the regulation of learning and memory as well as depression, anxiety and pain (Carvalho et al., 2010).

In rodents, both Δ^9-THC and other synthetic compounds alter specific types of behavior, including nociception, motor activity, memory, and feeding (Chaperon and Thiebot, 1999). It has been reported that cannabinoids modify rodent behavioral responses to novelty in different tests (Onaivi et al., 1990; Navarro et al., 1993; Giuliani et al., 2000).

Khat (*Catha edulis* F.) has been consumed for centuries by people living around the horn of Africa, East Africa and the Middle East (Connor et al., 2002; Carlini, 2003). Cathinone is the main psychoactive alkaloid of fresh khat leaves and has the same indirect sympathomimetic mechanism of action as amphetamine (AL-Hebshi and Skaug, 2005). The effects observed following khat consumption are generally of central stimulation and include euphoria, excitation, anorexia, increased respiration, hyperthermia, analgesia and increased sensory stimulation (Feyissa and Kelly, 2008). Khat has also been shown to increase locomotion and alter performance in several behavioral paradigms in rodents (Oyungu et al., 2007; Bedada and Engidawork, 2010). The emergence of "designer" drugs or "new psychoactive substances" (NPS) in Europe and USA that contain synthetic cathinones in "bath salts" and synthetic cannabinoids in "spice" are used and abused to mimic the effects of khat or marijuana, respectively (Baumann et al., 2014).

Several studies have shown the existence of an interaction between the eCB system and some drugs of abuse, such as opioids (Scavone et al., 2013), nicotine (Viverosa et al., 2006; Werling et al., 2009), alcohol (Ishiguro et al., 2007) and cocaine (Xi et al., 2012).

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In particular, CB_1R blockade reduces the behavioral effects of many drugs of abuse, including marijuana, morphine, ethanol, and nicotine (Balerio et al., 2006; Biala and Kruk, 2008). Khat is believed to interact with the monoaminergic system and the finding that khat reverses haloperidol induced but not morphine induced catalepsy (Geresu and Engidawork, 2010) lends support to this notion. So far, to the best of our knowledge, there is no study conducted to evaluate the interaction of cannabinoids and khat, although the two agents have the capacity to produce pleasant sensation. The aim of this study is therefore to evaluate the possible existence of behavioral interaction between the eCB system and khat after acute administration in mice.

2. Results

2.1. Locomotor activity

Locomotor activity (Fig. 1) was significantly associated with treatment groups [$F(5,30)=29.052$, $p<0.001$]. During post-hoc analysis, treatment of mice with khat 300 mg/kg (K300) significantly increased ($p<0.001$) locomotion by 76.2% compared to vehicle-treated (control) mice. Treatment with WIN55,212-2, a non-selective CBR agonist, did not, however, alter locomotion compared to control, but locomotion was significantly lower ($p<0.01$) than induced by K300. Interestingly, treatment with the combination of K300 and WIN55,212-2 produced locomotion that was significantly greater than the one produced by the individual agents as well as the vehicle. By contrast, treatment with khat and AM251 (a CB_1R antagonist) or AM630 (a CB_2R antagonist) significantly attenuated khat-induced hyper-locomotion by 31% ($p<0.001$) and 28.1% ($p<0.001$), respectively, and the value was brought down to the control level. In addition, extent of locomotion produced by the combination of khat and the antagonists was significantly lower than that observed with the combination of khat and the agonist.

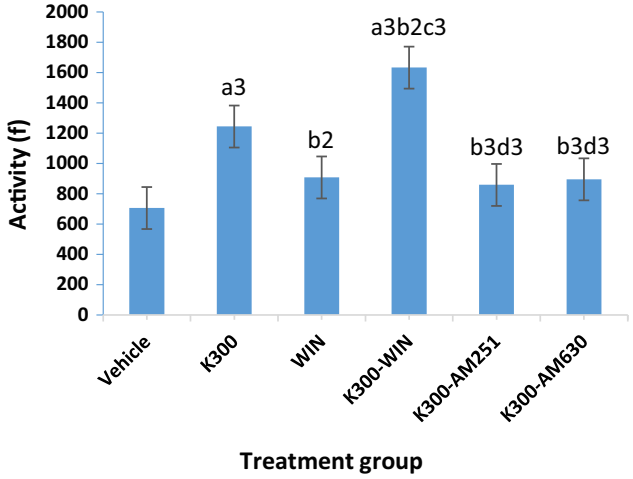

Fig. 1. Acute effect of khat and cannabinoid ligands on motor activity in mice: values are mean ± SEM (n=6). Statistical analysis was done using one way ANOVA followed by Tukey's multiple comparison test; a-compared to vehicle control, b-compared to K300, c- compared to WIN, d- compared to K300-WIN. $^3p<0.001$, $^2p<0.01$. f – frequency, vehicle=Tween 80, K300=khat extract 300 mg/kg, WIN=non selective cannabinoid receptor agonist 1 mg/kg, K300-AM251=khat extract 300 mg/kg followed by cannabinoid CB_1 selective antagonist 1 mg/kg, K300-AM630=khat extract 300 mg/kg followed by cannabinoid CB_2 selective antagonist 1 mg/kg, K300-WIN=khat extract 300 mg/kg followed by non selective cannabinoid receptor agonist 1 mg/kg.

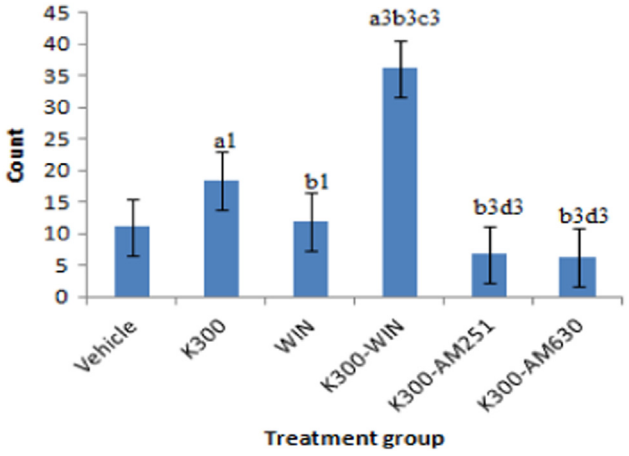

Fig. 2. Acute effect of khat and cannabinoid ligands on total arm entry in Y-maze: values are mean ± SEM (n=6). Statistical analysis was performed by one way ANOVA followed by Tukey's multiple comparison test; a-compared to vehicle control, b-compared to K300, c-compared to WIN, d-compared to K300-WIN. $^3p<0.001$, $^1p<0.05$. Vehicle=Tween 80, K300=khat extract 300 mg/kg, WIN=non selective cannabinoid receptor agonist 1 mg/kg, K300-AM251=khat extract 300 mg/kg followed by cannabinoid CB_1 selective antagonist 1 mg/kg, K300-AM630=khat extract 300 mg/kg followed by cannabinoid CB_2 selective antagonist 1 mg/kg, K300-WIN=khat extract 300 mg/kg followed by non selective cannabinoid receptor agonist 1 mg/kg.

2.2. Working memory related behavioral alternation

The total alley visit [$F(5, 30)=43.315$, $p<0.001$] as well as percent alternation [$F(5,30)=10.251$, $p<0.001$] in the Y-maze was significantly associated with the treatment group. Post-hoc analysis revealed that whilst K300 significantly increased total arm entry ($p<0.05$) (Fig. 2), it failed to alter percent alternation (Fig. 3) compared to controls in the Y-maze. On the other hand, WIN55,212-2 altered neither arm entry nor percent alternation compared to control mice. However, arm entry caused by WIN55,212–2 was significantly lower ($p<0.05$) than K300 but percent alternation was not different between the two agents.

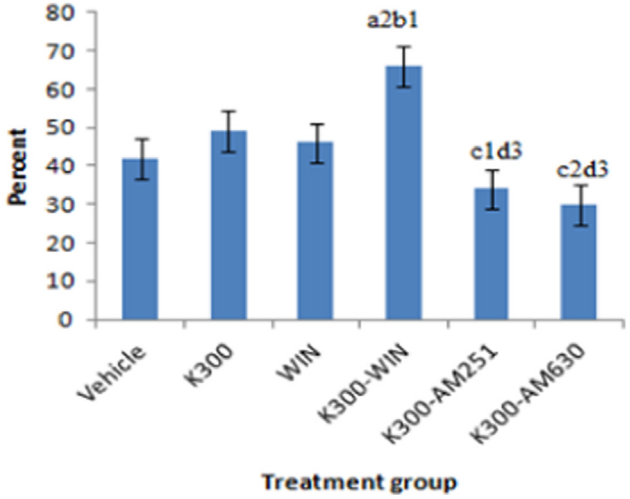

Fig. 3. Acute effect of khat and cannabinoid ligands on percentage alternation in Y-maze: values are mean ± SEM (n=6). Statistical analysis was performed by one way ANOVA followed by Tukey's multiple comparison test; a-compared to vehicle control, b-compared to K300, c-compared to WIN, d-compared to K300-WIN. $^3p<0.001$, $^2p<0.01$, $^1p<0.05$. Vehicle=Tween 80, K300=khat extract 300 mg/kg, WIN=non selective cannabinoid receptor agonist 1 mg/kg, K300-AM251=khat extract 300 mg/kg followed by cannabinoid CB_1 selective antagonist 1 mg/kg, K300-AM630=khat extract 300 mg/kg followed by cannabinoid CB_2 selective antagonist 1 mg/kg, K300-WIN=khat extract 300 mg/kg followed by non selective cannabinoid receptor agonist 1 mg/kg.