Pharmacological enhancement of cannabinoid CB₁ receptor activity elicits an antidepressant-like response in the rat forced swim test

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Abstract

These experiments aimed to assess whether enhanced activity at the cannabinoid CB₁ receptor elicits antidepressant-like effects. To examine this we administered 1 and 5 mg/kg doses of the endocannabinoid uptake inhibitor AM404; 5 and 25 µg/kg doses of HU-210, a potent CB₁ receptor agonist; 1, 2.5 and 5 mg/kg of oleamide, which elicits cannabinoidergic actions; 1 and 5 mg/kg doses of AM 251, a selective CB₁ receptor antagonist, as well as 10 mg/kg desipramine (a positive antidepressant control) and measured the duration of immobility, during a 5-min test session of the rat Porsolt forced swim test. Results demonstrated that administration of desipramine reduced immobility duration by about 50% and that all of AM404, oleamide and HU-210 administration induced comparable decreases in immobility that were blocked by pretreatment with AM 251. Administration of the antagonist AM 251 alone had no effect on immobility at either dose. These data suggest that enhancement of CB₁ receptor signaling results in antidepressant effects in the forced swim test similar to that seen following conventional antidepressant administration.

Keywords: Cannabinoid; Antidepressant; Forced swim test; Oleamide; Rat

1. Introduction

The endogenous cannabinoid (EC) system is a neuro-modulatory system in the brain that is responsive to the psychoactive constituent of cannabis, THC, as well as endogenously synthesized ligands, such as anandamide (AEA) and 2-arachidonylglycerol (2-AG). In addition to the endogenous ligands, this system is composed of a selective neural receptor (CB₁) as well as degradative enzymes such as fatty acid amide hydrolase (FAAH), which are responsible for catabolism of these ligands (Freund et al., 2003). While the physiological role of this system in the regulation of many processes, for example, feeding behavior (Harrold and Williams, 2003), is well documented, less is known about its role in mental and cognitive functioning. Evidence has begun to accumulate suggesting that the EC system has a functional role in the expression of emotional behavior (Martin et al., 2002). The basis for this claim is found in epidemiological research documenting that recreational or medicinal consumption of cannabis in humans, which functionally activates the EC system, typically results in an elevation in mood, the induction of euphoria and a reduction in stress, anxiety and depressive symptoms (Green et al., 2003; Gruber et al., 1996; Williamson and Evans, 2000). These findings are paralleled by animal research that has shown that administration of low doses of CB₁ receptor agonists or inhibitors of the FAAH enzyme, which elevate AEA, reduces anxiety-like behavior (Berrendero and Malдонado, 2002; Hill and Gorzalka, 2004; Kathuria et al., 2003). Of particular interest is the research that has revealed altered emotional behavior in mice genetically bred to lack the CB₁ receptor. These animals have been shown to display an enhancement of anxiety in behavioral tests such as the light–dark box and the elevated plus maze, as well as an increased susceptibility to the anhedonic effects of chronic stress (Haller et al., 2002; Martin et al., 2002). From this one
can conclude that mild activation of the EC system results in anxiolysis and the expression of positive emotions, whereas deficits in this system result in increased anxiety and depressive-like behaviors.

The implication of this theory of emotional regulation by the EC system raises the possibility that the EC system itself may play a functional role in the manifestation of various affective disorders, especially major depression. The symptomatology of depression is typically characterized by depressed mood, anhedonia (loss of interest in rewarding stimuli such as those associated with sexual activity) and extreme alterations in vegetative functions (i.e., insomnia or hypersomnia, decreased appetite or increased appetite; American Psychiatric Association, 1994). Furthermore, many of the systems that are dysregulated in depression are also influenced by EC activity. For example, EC activity has been shown to be involved in the maintenance of feeding behavior (Harrold and Williams, 2003); thus deficiencies in this system could result in the decreased appetite and reduced body weight seen in some cases of depression. Furthermore, AEA has been suggested to be integral to the maintenance of the sleep–wake cycle, as administration of AEA can induce sleep (Murillo-Rodriguez et al., 2003) and pharmacological blockade of EC activity results in increased wakefulness (Santucci et al., 1996). As with feeding behavior, deficits in this system could result in the insomnia seen in some cases of depression. There is increasing evidence that the EC system is important for activation of reward circuitry in the brain as pharmacological blockade of the CB1 receptor results in an attenuated response to both pharmacological and naturally rewarding stimuli (Chaperon et al., 1998; Arnone et al., 1997). Thus, a deficiency in EC activity could lead to a blunting of responsiveness to rewarding stimuli, which is one of the core components of depression.

Biochemical evidence also supports the idea of an EC deficit in depression. For example, chronic stress, which is known to be a predictor of the onset of depressive episodes, downregulates both the CB1 receptor as well as EC content in the hippocampus, implying that the EC system is environmentally sensitive and may become turned off during times of prolonged stress (Hill et al., 2005). Furthermore, major depression is frequently characterized by a deficit in serotonergic (5-HT) activity in the brain (Owens and Nemeroff, 1994) and it has been suggested that 5-HT may play a role in the ability of the CB1 receptor to couple to its G-protein second messenger system, and thus launch a cellular response (Devlin and Christophoulus, 2002). Consistent with this, deficiencies in central serotonergic activity have been shown to result in a desensitization of the GTP\(\gamma\)S signaling cascade activated by the CB1 receptor, as well as an attenuation of the behavioral response to administration of a CB1 receptor agonist (Overbury et al., 2003). Furthermore, chronic treatment with the serotonin reuptake inhibitor fluoxetine, which elevates synaptic serotonin concentrations, induces a hypersensitization of GTP\(\gamma\)S signaling elicited by CB1 receptor activation (Olivia et al., 2003). This enhancement of CB1 receptor signaling through chronic administration of fluoxetine also suggests that antidepressants may act to increase EC activity, demonstrating a possible bidirectional association between stress and antidepressant treatment on the endocannabinoid system.

Since EC activity seems to be downregulated by both exposure to stress and deficiencies in serotonin, and the behavioral effects of deficient EC activity mirror those seen in depression, it is possible that a deficit in EC activity may be important for the expression of major depression. Accordingly, enhancement of this system may be a novel pathway for the pharmacotherapy of depression. The next logical step in examining this hypothesis is to test the effects of activation of this system alone in animal models predictive of antidepressant efficacy. To date, one of the most effective preclinical animal models in use is the forced swim test (FST; Cryan et al., 2002). This series of experiments was designed to examine the effect of pharmacological enhancement and blockade of the endocannabinoid system on behaviors in the FST.

2. Experimental procedures

2.1. Animals and housing

Male Long-Evans rats that were 10 weeks of age and weighed between 300 and 350 g were employed in this study. All subjects were housed in groups of three in triple mesh wire cages in a colony room that had a maintained temperature of 21 ± 1 °C and a reverse 12:12-h light–dark cycle (lights off at 0900h). All rats had ad libitum access to tap water and Purina Rat chow and were handled 4 times a week prior to testing. All experimental testing on animals was in accordance with the Canadian Council of Animal Care and the Animal Care Ethics Committee of the University of British Columbia.

2.2. Drugs

All drugs used in this study were obtained from Tocris Cookson Ltd. (Bristol, UK), except for oleamide (cis-9,10-octadecanamide) and desipramine (5H-dibenzo[b,f]azepine-5-propanamine, 10,11-dihydro-N-methyl-6-endo-dimethylheptyl), which were obtained from Sigma-Aldrich. Oleamide, AM404 (N-(4-hydroxyphenyl)-5Z,8Z,11Z,14Z-eicosatetraenamide), AM 251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide) and HU-210 ([3R, 4R]-7-hydroxy-\(\Delta^2\)-tetrahydrocannabinol 1,1-dimethylheptyl) were each dissolved in a 1:1:18 solution of dimethyl sulfoxide/Tween 80/0.9% saline. Desipramine was dissolved in 0.9% saline. AM404 and AM251 were injected at doses of 1 mg/kg and 5 mg/kg, HU-210 was injected...
at doses of 5 and 25 μg/kg, oleamide was injected at doses of 1, 2.5 and 5 mg/kg and desipramine was administered at a dose of 10 mg/kg. Injections were given intra-peritoneally (i.p) at a concentration of 1 ml/kg using 26 gauge 1/2” stainless steel needles. Control subjects were given vehicle injections.

2.3. Apparatus

Plexiglas cylindrical containers (diameter 35 cm and height 45 cm) were used during forced swim testing. Given the documented influence of water depth on FST behaviors (Abel, 1994), each container was filled to 30 cm so that an animal could only touch the bottom with the tip of its tail, and water temperature was maintained at a constant 23±1 °C. To remove the influence of potential alarm substances on behaviors in the FST (Abel, 1991), fresh water was introduced prior to each test. All test sessions were recorded with a video camera (Hitachi 2500A) positioned such that the entire container was in full sight, and videotapes of test sessions were subsequently scored by blind, trained observers.

2.4. Procedure

All behavioral testing occurred during the middle third of the animals’ dark cycle. This protocol was chosen because previous research has determined that rats display higher levels of immobility during the dark phase (Kelliher et al., 2000), thus allowing for easier determinations of reductions in immobility. Consistent with the modified method of testing in the FST, animals were subjected to two swim sessions (Porsolt et al., 1978; Cryan et al., 2002). The first swim session was composed of a 15-min pre-exposure session, after which the rats were removed with the assistance of a wire mesh ladder. Rats were then placed in maternity bins, dried off with disposable towels and returned to their home cages. Twenty four hours later all subjects were subjected to a test swim session of 5 min in duration. During the test session the duration of immobility, defined as when the rat was stationary and only made the minimal movements necessary to stay afloat, was measured.

Consistent with the previous research assessing the antidepressant potential of various agents, the drugs in this study were administered three times between the two swim sessions, at 23.5, 5 and 1 h prior to the test session (Cryan et al., 2002). For dual injection tests, AM 251 was administered 10 min prior to AM 404, HU-210 and oleamide at all time points.

2.5. Statistics

A one-way analysis of variance was used to analyze the behavioral data obtained from the FST. Post hoc analysis was performed using a Tukey’s test, and all significance levels were set at a $p$ value of 0.05.

3. Results

In this experiment, desipramine (10 mg/kg) significantly reduced immobility in the FST [$t(12)=4.41, p=0.001$]. The anti-immobility effects of desipramine can be seen in Fig. 1.

Fig. 1. The effect of desipramine (DES) on the occurrence of immobility in the FST. Data are presented as mean duration±S.E.M. Differences that were significant at $p<0.05$ are denoted by *.

Fig. 2. The effect of AM404 administration on the occurrence of (a) immobility in the FST; (b) the effect of AM 251 pretreatment on the anti-immobility effects of AM 404 in the FST. Data are presented as mean duration±S.E.M. All differences from the control group that were significant at $p<0.05$ are denoted by *.
Administration of AM404 significantly reduced immobility \([F(2, 16)=4.53, p=0.028]\), with post hoc analysis revealing that the reduction in immobility was significant at 5 mg/kg \((p=0.022)\), but not at 1 mg/kg \((p=0.26)\). Pretreatment with 1 mg/kg of the selective CB1 receptor antagonist AM 251 prevented the reduction in immobility induced by 5 mg/kg of AM 404 \([F(2, 16)=6.28, p=0.01]\), and post-hoc analysis revealed that 5 mg/kg of AM 404 alone significantly reduced immobility \((p<0.01)\). The AM 404 group pretreated with AM 251 did not differ from the control group \((p=0.17)\). Data regarding the effects of oleamide on immobility can be viewed in Fig. 2a. Pretreatment with the selective CB1 receptor antagonist AM 251 prevented the significant reduction in immobility induced by 5 mg/kg of oleamide \([F(2, 20)=2.42, p>0.05]\), in that the group treated with both 5 mg/kg oleamide and 1 mg/kg AM 251 no longer differed from the control group \((p=0.30)\). The data regarding the blockade of the anti-immobility effects of oleamide by AM 251 can be seen in Fig. 4b.

AM 251 administered alone at a dose of 1 mg/kg and 5 mg/kg did not affect time spent immobile \([F(2, 18)=0.69, p=0.014]\, such that 25 μg/kg of HU-210 elicited a reduction in immobility as compared to the control group \((p=0.02)\) that was not seen following pretreatment with AM 251 \((p=0.60)\). The effects of HU-210 on immobility in the FST and its reversal by pretreatment with AM 251 can be seen in Fig. 3a and b, respectively.

Oleamide elicited a significant, dose-dependent reduction in immobility \([F(3, 27)=3.74, p<0.05]\). Post hoc analysis revealed that the reduction elicited by oleamide was significant at both the 2.5 \((p=0.02)\) and 5 mg/kg dose \((p=0.03)\), but not at the 1 mg/kg dose \((p>0.05)\). Data regarding the effects of oleamide on immobility can be viewed in Fig. 4a. Pretreatment with the selective CB1 receptor antagonist AM 251 prevented the significant reduction in immobility induced by 5 mg/kg of oleamide \([F(2, 20)=2.42, p>0.05]\, in that the group treated with both 5 mg/kg oleamide and 1 mg/kg AM 251 no longer differed from the control group \((p=0.30)\). The data regarding the blockade of the anti-immobility effects of oleamide by AM 251 can be seen in Fig. 4b.
were no significant differences between groups.

The reuptake of dopamine, norepinephrine and serotonin by the reuptake pump (Bass et al., 2004) is mediated by the CB1 receptor, which elicits an antidepressant-like effect in this preclinical paradigm.

*p > 0.05*. Data for the effects of AM 251 administration on immobility appear in Fig. 5.

### 4. Discussion

These data indicate that pharmacological enhancement of CB1 receptor activity elicits an antidepressant effect in the rat forced swim test, as demonstrated by significant reductions in immobility. The activity of CB1 receptor antagonists (Banerjee et al., 1975; Steffens and Feuerstein, 2004), suggesting that they may have a mechanism of action similar to that of tricyclic antidepressants, such as desipramine. However, other evidence has demonstrated that cannabinoids also possess the ability to inhibit serotonin release in cortical slices (Nakazi et al., 2000) and pharmacological antagonism of CB1 receptors results in cortical serotonin and norepinephrine release (Tzavara et al., 2003). Thus, further biochemical work is required to ascertain which similarities between the mechanism of action of conventional antidepressants and the pharmacological actions of cannabinoids may mediate this response.

Oleamide, like AM404 and HU-210, resulted in a dose-dependent reduction in immobility. These data are in line with recent research demonstrating that sleep deprivation decreases immobility in the forced swim test (Lopez-Rodriguez et al., 2004), as oleamide concentrations are known to elevate following sleep deprivation (Lerner et al., 1994). The anti-immobility effect of oleamide in this study was found to be sensitive to blockade of CB1 receptors, suggesting that this effect was mediated by engagement of the EC system. Oleamide is known to act as a competitive inhibitor of FAAH (Mechoulam et al., 1997; Lichtman et al., 2002), the enzyme responsible for EC degradation, thus the effects demonstrated in this study could be due to elevations in EC concentration. It should be noted that this entourage effect of oleamide on anandamide hydrolysis requires high levels of oleamide (Mechoulam et al., 1997). The doses used in this study were relatively low, however, the administration of oleamide three times within a 24-h period in this study may have produced high enough concentrations of oleamide to reduce hydrolysis of anandamide. Alternatively, it has been shown that oleamide has the ability to interact directly with the CB1 receptor in vitro (Leggett et al., 2004), however this is in contrast to the previous reports demonstrating the opposite finding (Mechoulam et al., 1997; Lichtman et al., 2002). Despite this controversy, the possibility does exist that oleamide may be acting as a direct CB1 receptor agonist in vivo. Together, these findings suggest that oleamide elicits a robust antidepressant-like effect in the FST that is similar to that seen following sleep deprivation, and that the EC system is involved in this effect. An interesting follow-up to this study would be to examine directly if the antidepressant effects of sleep deprivation are mediated by oleamide, or through interactions with the EC system.

Interestingly, administration of the selective CB1 receptor antagonist AM251 did not affect the presence of immobility in the FST, an unanticipated finding. It should be noted though that the lack of effect on immobility found here contrasts with findings from other research. Recently it was shown that administration of either AM251 or SR 141716A, another selective CB1 receptor antagonist, had an antidepressant effect in the mouse forced swim test (Shearman et al., 2003; Tzavara et al., 2003). However, the mouse FST is fundamentally different in design (it involves only a single

![Fig. 5. The effect of AM 251 administration on the occurrence of immobility in the FST. Data are presented as mean duration ± S.E.M. There were no significant differences between groups.](image-url)
swim trial), suggesting three major possibilities to explain this difference. First, many drugs are known to elicit differential responses in the rat versus the mouse FST (Borsini and Meli, 1988). Second, the rat FST is largely based on a learned response where the animals learn that active behaviors do not facilitate their escape, whereas in mice the test simply measures their response to novel drug administration (Borsini and Meli, 1988; Cryan et al., 2002). Third, the possibility exists that EC signaling could elicit a biphasic effect on depressive-like behaviors in the same manner as it does for anxiety-like behavior. For example, HU-210 is anxiolytic at low doses and anxiogenic at high doses (Hill and Gorzalka, 2004; Marco et al., 2004) and both CB1 receptor agonists and antagonists have been shown to be anxiogenic and anxiolytic (Berrendero and Maldonado, 2002; Navarro et al., 1997; Akinshola et al., 1999), reflecting the fact that changes in EC signaling may be both antidepressant-like and depressive-like, depending on factors such as testing conditions, strain and species of animal and drug dose.

The present data along with data obtained from other laboratories suggest that enhanced EC activity occurs in many regimens that elicit an antidepressant response. Both sleep deprivation and environmental enrichment (Porsolt et al., 1978; Lopez-Rodriguez et al., 2004) are known to elicit positive responses in the FST. Furthermore, environmental enrichment elevates anandamide concentrations (Wolf and Matzinger, 2003), and sleep deprivation increases levels of oleamide (Lerner et al., 1994), which then acts as a competitive substrate for FAAH and prevents AEA catalysis (Mechoulam et al., 1997), suggesting that the reduction in immobility in the FST seen following these regimens may be due in part to enhanced EC activity. This idea is substantiated by the present data demonstrating that oleamide elicits a CB1 receptor-dependent reduction in immobility in the FST. The results of this study demonstrate that enhancement of EC activity alone is sufficient to elicit an antidepressant response. This hypothesis is further supported by a knowledge that activation of this system results in mood enhancement (Green et al., 2003) and antidepressant effects (Williamson and Evans, 2000) in humans, and that suppression of this system can induce anxiogenic and depressive-like effects in non-human species (Martin et al., 2002).

One aspect of the EC system that makes it particularly advantageous for the treatment of depression is its ability to elicit fast acting responses. For example, current antidepressant therapies such as treatment with fluoxetine require 2–6 weeks to become therapeutically effective (Thase et al., 2001). Pharmacological agents which influence EC activity, such as AM404, are known to have very fast acting behavioral effects (Gonzalez et al., 1999). This is supported by knowledge that cannabis consumption in humans results in behavioral and mood alterations within a matter of minutes (Ohlsson et al., 1980). Therefore, in addition to their ability to elicit antidepressant responses, drugs which target the EC system may also have a significant advantage in the pharmacotherapy of affective disease because of their fast acting potential. Furthermore, the apparent anxiolytic properties elicited by FAAH inhibition (Kathuria et al., 2003) also suggest that this avenue is advantageous because of the potential to treat both anxiety and depression. However, due to the high frequency of aversive reactions to cannabis in depressed patients (Ablon and Goodwin, 1974) inhibition of EC degradation may be a more suitable target for antidepressant treatment than cannabis itself, especially for subtypes of major depression, such as melancholic depression, which are characterized by hypophagia, insomnia, anhedonia and anxiety (Gold and Chrousos, 2002).

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