PHARMACOLOGICAL ENHANCEMENT OF CANNABINOID TYPE 1(CB_1) RECEPTOR ACTIVIY ELICITS AN ANTIDEPRESSANT-LIKE EFFECT IN THE RAT FORCED SWIM TEST

by

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ABSTRACT

Accumulating evidence suggests that the endocannabinoid system may play a role in emotional regulation, and thus may be a novel target for antidepressant pharmacotherapeutics. This experiment aimed to assess whether enhanced CB_1 receptor activity possesses antidepressant properties. To examine the effect of modulation of cannabinoid activity on behaviors in the rat Porsolt forced swim test, we administered 1 and 5 mg/kg doses of the endocannabinoid uptake inhibitor AM404; 1, 2.5 and 5 mg/kg of the sleep-inducing lipid oleamide, which may possess cannabinoidergic properties; 5, 10 and 25 ug/kg doses of HU-210, a potent CB_1 receptor agonist; and 1 and 5 mg/kg doses of AM 251, a selective CB_1 receptor antagonist, and scored the expression of immobility, swimming and struggling during a 5 min test session. Administration of AM404 caused a dose dependent decrease in immobility that was blocked by pretreatment with AM 251. Oleamide also elicited a dose dependent decrease in immobility that was similarly prevented by pretreatment with AM251. Administration of the antagonist AM 251 alone had no effect on immobility at either dose. Furthermore, the agonist HU-210, at doses of 5 and 25 ug/kg, elicited a reduction in immobility. These data suggest that enhancement of CB_1 receptor signaling results in antidepressant effects in the forced swim test, and that future research should determine whether elevation of endogenous cannabinoids would be a suitable target for the pharmacotherapy of affective and stress related disorders.

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LIST OF NOMENCLATURE AND ABBREVIATIONS

hr: Hour

kg: Kilogram

mg: Milligram

min: Minute

ml: Millilitre

s: Second

2-AG: 2-Arachidonylglycerol

5-HT: 5-Hydroxytryptamine (Serotonin)

5-HT_{2A}: Serotonin Type 2A Receptor

AEA: Arachidonylethanolamine (Anandamide)

CB_1: Cannabinoid Type 1 Receptor

EC: Endocannabinoid

FAAH: Fatty Acid Amide Hydrolase

FST: Forced Swim Test

GTPγS: Guanosine Thiotriphosphate

OLE: Oleamide

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Introduction

The endogenous cannabinoid (EC) system is a neuromodulatory 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 (CB_1) 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 this system plays in the regulation of many processes, for example, feeding behavior (Harrold and Williams, 2003), is well documented, less is known about the role it plays 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 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 in those using it for medicinal purposes (Green et al., 2003; Williamson and Evans, 2000). These findings are paralleled by animal research that has shown that administration of low doses of CB_1 receptor agonists or inhibitors of the FAAH enzyme, which elevate AEA, results in a reduction in anxiety-like behavior (Berrendero and Maldonado, 2002; Kathuria et al., 2003). Of particular interest is research that has demonstrated alterations in emotional behavior in mice genetically bred to lack the CB_1 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 (Murrillo-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 CB_1 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, has recently been shown to result in a downregulation of both the CB_1 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., 2003). Furthermore, major depression is frequently characterized by a deficit in serotonergic (5-HT) activity in the brain (Owens and Nemeroff, 1994). In accordance with this, it has been suggested that 5-HT may play a role in the ability of the CB_1 receptor to couple to its G-protein second messenger system, and thus launch a cellular response (Devlin and Christopolous, 2002). Consistent with this, deficiencies in central serotonergic activity have been shown to result in a desensitization of the GTPγS signaling cascade activated by the CB_1 receptor, as well as an attenuation of the behavioral response to administration of a CB_1 receptor agonist (Overbury et al., 2003). Furthermore, chronic treatment with the serotonin reuptake inhibitor fluoxetine, which results in enhanced synaptic serotonin concentrations, induces a hypersensitization of GTPγS signaling elicited by CB_1 receptor activation (Olivia et al., 2003).

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 tempting to assume that a deficit in EC activity may be important for the expression of major depression, and accordingly enhancement of this system may be a novel pathway for the pharmacotherapy of depression. As previously stated,

treatment with chronic fluoxetine, which is currently the most clinically effective antidepressant, results in a supersensitization of the CB_1 receptor (Olivia et al., 2003). Moreover, many non-pharmacological treatments for depression also result in elevated EC activity. For example, environmental enrichment, which elicits antidepressant responses in animals (Porsolt et al., 1978), results in a ten fold elevation in hippocampal anandamide content (Wolf and Matzinger, 2003). Furthermore, sleep deprivation, one of the few effective fast acting antidepressant regimens (Adrien, 2002), results in elevations of the lipid oleamide (Lerner et al., 1994), whose primary actions involve inhibition of FAAH and subsequent elevations in AEA content (Mechoulam et al., 1997). Similarily, voluntary exercise, which has also been shown to have mood elevating properties, results in enhanced EC levels (Sparling et al., 2003). This demonstrates that across both pharmacological and non-pharmacological treatments for depression, enhancement of the EC system appears to be a common link.

The next logical step in examining these hypotheses is to investigate the effects of activation of this system alone in animal models predictive of antidepressant efficacy. To date, the most effective animal model in use is the forced swim test (FST; Cryan et al., 2002). This test consists of exposing a rat to an inescapable swim session for fifteen minutes, after which it is removed and then re-exposed twenty four hours later for a five minute test session (Cryan et al., 2002). During the twenty four hour interval between swim sessions, the rat is exposed to a series of three administrations of the drug of investigation (Cryan et al., 2002). During the test session, untreated animals typically spend a large proportion of time immobile, but also display some active behaviors such as swimming and struggling (Cryan et al., 2002). Animals which have been treated with

the antidepressant regimen display a lower frequency of immobility and a higher level of active, escape directed behaviors (Cryan et al., 2002). The validity of this test has been supported by findings that it is responsive to both pharmacological (approx. 90% of clinically effective antidepressants elicit true positive responses; Borsini and Meli, 1988) and non-pharmacological [sleep deprivation (Lopez-Rodriguez et al., 2004), electro-convulsive shock (Kawashima et al., 1987) and environmental enrichment (Porsolt et al., 1978) all elicit true positive responses] antidepressant regimens. Additionally, it has also been demonstrated that "depressive" phenomena that are associated with enhanced dysphoria such as chronically elevated glucocorticoids (Hill et al., 2003), post-partum depression (Galea et al., 2001) and amphetamine withdrawal (Cryan et al., 2003), result in an increased expression of immobility. Furthermore, repeated administrations of antidepressant drugs over relatively short periods of time (i.e., inter-swim session interval) are believed to result in neuroadaptive changes similar to those seen following long-term, chronic treatment (Cryan et al., 2002). Thus while the FST may not be a suitable model for exploring symptomatic and emotional changes in depression, it does appear to be a useful tool in examining the efficacy of antidepressant agents.

The present investigation sought to examine the effect of pharmacological enhancement and blockade of the endocannabinoid system on behaviors in the FST. To examine this, we first tested the effect of pharmacological elevation of endocannabinoids via inhibition of endocannabinoid catabolism using the endocannabinoid uptake inhibitor AM404. We also examined the dose dependent effects of the endogenous lipid oleamide which possess FAAH inhibitor capabilities and thus elevates endogenous cannabinoid activity. To further examine the effect of CB_1 receptor activation specifically, we

administered varying doses of the selective CB_1 receptor agonist HU-210. Finally, we examined the effect of CB_1 receptor blockade, using the selective antagonist AM251, on behaviors in the FST. This research demonstrated that enhancement of endocannabinoid activity through AM404, oleamide or HU-210 reduced immobility and elicited an antidepressant effect. This suggests that the endocannabinoid system may be a suitable target for the development of a novel class of antidepressant drugs.

Methods

Subjects

Male Long-Evans rats that were 10 weeks of age and weighed between 300-350 g were used 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 12h:12h 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 within accordance of the Canadian Council of Animal Care and the Animal Care Ethics Committee of the University of British Columbia.

Drugs

All drugs used in this study were obtained from Tocris Cookson Ltd. (Bristol, UK), except for oleamide (cis-9,10-Octadecanamide), which was obtained from Sigma-Aldrich. Oleamide (cis-9,10-Octadecanamide), AM404 (*N*-(4-Hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide), AM 251 (*N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) and HU-210 ([3R, 4R]-7-hydroxy-Δ^6-tetrahydrocannabinol 1,1-dimethylheptyl) were each dissolved in a 1:1:18 solution of dimethyl sulfoxide: Tween 80: 0.9% saline. AM404 and AM251 were

injected at doses of 1mg/kg and 5 mg/kg, HU-210 was injected at doses of 5, 10 and 25 μg/kg and oleamide was injected at doses of 1, 2.5 and 5 mg/kg. Injections were given intra-peritoneally (i.p) at a concentration of 1 ml/kg using 26 gauge ½" stainless steel needles. Control subjects were given vehicle injections.

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.

Procedure

Consistent with the modified method of testing in the FST, animals were subjected to two swim sessions (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 hr later all subjects were subjected to a test swim session of 5 min in duration. During the test session, immobile and active behaviors were measured. Immobility was defined as when the rat was stationary and only made the minimal movements necessary to stay afloat; swimming

was defined as when the rat was actively moving at least two limbs to induce active motion around the swim chamber; struggling was defined as when the rat was thrashing with its forepaws above the water surface and was in a vertical position (Detke et al., 1995). The duration of each of these behaviors during the test session was measured.

Consistent with 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 hr prior to the test session (Cryan et al., 2002). For dual injection tests, AM 251 was administered 10 min prior to AM 404 and oleamide at all time points.

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 .05.

Results

Results from this experiment demonstrated that pharmacological enhancement of cannabinoid CB_1 receptor activity reduced immobility in the FST. Administration of AM404 was found to significantly reduce immobility [$F(2, 16) = 4.53$, $p = .028$], with post hoc analysis revealing that the reduction in immobility was significant at 5 mg/kg ($p = .022$), but not at 1 mg/kg ($p = .26$). This reduction in immobility was accompanied by a significant increase in swim time [$F(2, 16) = 6.81$, $p < .01$], that was again only seen at the 5 mg/kg dose ($p < .01$), whereas time spent struggling was unchanged [$F(2, 16) = 1.88$, $p > .05$]. The effects of AM404 on immobility, swimming and struggling can be seen in Fig. 1a, 1b and 1c, respectively.

Pretreatment with 1mg/kg of the selective CB_1 receptor antagonist AM 251 prevented the reduction in immobility induced by 5 mg/kg of AM 404 [$F(2, 16) = 6.28$, $p = .01$], with post-hoc analysis determining that 5 mg/kg of AM 404 alone significantly reduced immobility ($p < .01$). The AM 404 group pretreated with AM 251 did not differ from the control group ($p = .17$). Furthermore, pretreatment with AM 251 completely occluded the enhancement of swimming elicited by AM 404 [$F(2, 16) = 11.03$, $p = .001$]. Post hoc analysis revealed that 5 mg/kg AM 404 alone induced a significant increase in amount of time spent swimming ($p = .001$). The AM 404 group pretreated with AM 251 did not differ from the control group ($p = .58$).The reversal of the effects of AM 404 by AM 251 on immobility and swimming behavior can be viewed in Fig. 2a and 2b.

Oleamide elicited a significant, dose dependent reduction in immobility [$F(3, 27) = 3.74$, $p < .05$]. Post hoc analysis revealed that the reduction elicited by oleamide was significant at both the 2.5 ($p = .02$) and 5 mg/kg dose ($p = .03$), but not at the 1 mg/kg dose ($p > .05$). Data regarding the effects of oleamide on immobility can be viewed in Fig. 3a. This significant change in immobility however, was not accompanied by any significant changes in either swimming [$F(3, 27) = 1.02$, $p > .05$] or struggling [$F(3, 27) = 1.32$, $p > .05$]. The effects of oleamide on swimming and struggling can be seen in Fig. 3b and 3c.

Pretreatment with the selective CB_1 receptor antagonist AM 251 prevented the significant reduction in immobility induced by 5 mg/kg of oleamide [$F(2, 20 = 2.42)$, $p > .05$], in that the group treated with both 5 mg/kg oleamide and 1 mg/kg AM 251 no longer differed from the control group ($p = .30$). The data regarding the blockade of the anti-immobility effects of oleamide by AM 251 can be seen in Fig. 4. There were no

significant alterations in either swimming [$F(2, 20) = 2.67$, $p > .05$] or struggling [$F(2, 20) = 3.11$, $p > .05$] induced by the co-administration of these pharmacological agents (data not shown).

AM 251 administered alone at a dose of 1 mg/kg and 5 mg/kg did not affect time spent immobile [$F(2, 18) = 0.69$, $p > .05$]. However, AM 251 did significantly affect active behaviors. Specifically, it was found that the occurrence of swimming behavior was reduced following AM 251 treatment [$F(2, 18) = 5.46$, $p = .014$], with post-hoc analysis demonstrating that this reduction was only seen following administration of 5 mg/kg ($p = .05$), but not following 1 mg/kg of AM 251 ($p = .82$). Additionally, AM 251 also influenced the occurrence of struggling behavior [$F(2, 18) = 4.54$, $p = .025$], with post-hoc analysis demonstrating that struggling was increased following 5 mg/kg of AM 251 ($p = .04$), but not after 1 mg/kg ($p = .99$). Data for the effects of AM 251 administration on immobility, swimming and struggling appear in Fig 5a, 5b and 5c, respectively.

To further investigate the effects of CB_1 receptor stimulation on behaviors in the FST, we examined the effect of the highly selective and potent CB_1 agonist HU-210. As with AM 404, HU-210 administration was found to significantly affect the duration of time spent immobile [$F(3, 24) = 3.73$, $p = .025$); however post hoc analysis illustrated that this change was only seen in animals treated with either the 5 μg/kg ($p = .05$) or 25 μg/kg dose ($p = .03$) of HU-210, but not following the 10 μg/kg dose ($p > .05$). However, in contrast to AM 404 which also enhanced the occurrence of swimming behavior, HU-210 administration had no significant effect on either the occurrence of swimming

behavior [$F(3, 24) = 1.33$, $p > .05$] or struggling behavior [$F(3, 24) = 0.48$, $p > .05$]. The effects of HU-210 on behaviors in the FST are presented in Fig. 6.

Discussion

These data indicate that pharmacological enhancement of CB_1 receptor activity elicits an antidepressant effect in the rat forced swim test. Specifically, treatment with AM404, which has been shown to prevent cellular accumulation of endocannabinoids (Beltramo et al., 1997), resulted in a dose dependent decrease in the expression of immobility. This decrease in immobility was accompanied by a selective increase in swimming behavior, with no significant changes in struggling behavior. Both the reduction in immobility and enhancement of swimming behavior induced by AM 404 were prevented by pretreatment with the selective CB_1 receptor antagonist AM 251, indicating that this effect was likely mediated by the CB_1 receptor. Recent research has suggested that the neurochemical mechanisms mediating struggling and swimming behavior can be dissociated and are representative of engagement of the noradrenergic and serotonergic systems, respectively (Detke et al., 1995). Based on this assumption, it is reasonable to assume that the effects of AM404 are due to endocannabinoid interactions with the serotonergic system as revealed by its selective effect on swimming behavior. Interestingly, previous research has demonstrated that exogenous cannabinoids, such as THC, possess the ability to inhibit the reuptake of dopamine, norepinephrine and serotonin (Banerjee et al., 1975), suggesting that they may have a mechanism of action similar to that of tricyclic antidepressants. 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 CB_1 receptors results in cortical

serotonin and norepinephrine release (Tzavara et al., 2003). Thus, further biochemical work is required to ascertain what similarities between the mechanism of action of conventional antidepressants and the pharmacological actions of cannabinoids may mediate this response.

To further assess the role of endocannabinoids in the forced swim test, we examined how oleamide affected the expression of immobility. Oleamide, like AM404, resulted in a dose dependent reduction in immobility, however oleamide did not alter either swimming or struggling behavior, suggesting that the mechanism of action may be different for these two compounds. These data are in line with recent research that has demonstrated that sleep deprivation reduces 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). Furthermore, this also suggests that since the effects of oleamide and sleep deprivation in the forced swim test are comparable, that oleamide may be the active molecule in the antidepressant-like effect of sleep deprivation.

The anti-immobility effect of oleamide in this study was found to be sensitive to blockade of CB_1 receptors, suggesting that this effect was mediated by engagement of the endocannabinoid system. Oleamide is known to act as a competitive inhibitor for FAAH (Mechoulam et al., 1997; Lichtman et al., 2002), the enzyme responsible for endocannabinoid degradation, thus the effects demonstrated in this study could be due to elevations in endocannabinoid concentration. It should be noted that this entourage effect of oleamide on anandamide hydrolysis requires high levels of oleamide to be present (Mechoulam et al., 1997). The doses used in this study were relatively low, however, the administration of oleamide three times within a twenty four hour period in this study may

have produced high enough concentrations of oleamide to reduce hydrolysis of anandamide. Together with the CB_1 receptor sensitive anti-immobiolity effect found for AM404, these findings suggest that increasing the central endocannabinoid tone results in antidepressant-like effects in rats.

Examination of the putative effect of CB_1 receptor activity in the FST demonstrated that the selective CB_1 receptor agonist HU-210 mimicked the effects of AM404 by reducing the occurrence of immobility, however like oleamide HU-210 did not affect the occurrence of swimming as AM 404 did. It is not obvious why AM404 elicited a different profile from both oleamide and HU-210, since all of these effects appear to be CB_1 receptor-mediated. The most likely explanation of the difference between HU-210 and AM404 is that administration of a direct agonist would not be selective to a specific brain region since it would be administered systemically and have access to all structures. The inhibition of the cellular accumulation of endocannabinoids with AM 404 though, would simply result in a potentiation of the effects of endogenous ligands, which would not be synthesized and released uniformly across the brain. Thus, the behavioral differences between HU-210 and AM 404 documented here, may occur because of site-specific differences in CB_1 receptor activity in the brain induced by these two pharmacological agents. Furthermore, the behavioral profile suggests that endocannabinoids may be more selective than direct agonists in modulating serotonergic activity. However, this explanation does not explain why the effects of oleamide are more comparable to those of HU-210 than AM404. Recently, it has been shown that oleamide has the ability to interact directly with the CB_1 receptor *in vitro* (Leggett et al., 2004), however this is in contrast to 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 CB_1 receptor agonist *in vivo*, which would explain the discrepancy in these findings.

Administration of the selective CB_1 receptor antagonist AM251 did not affect the presence of immobility in the FST, an unanticipated finding. However, it did result in a shift in the expression of active behaviors, reducing the occurrence of swimming behavior and enhancing the presence of struggling behavior. This behavioral shift however, may simply be a byproduct of the known motor stimulating properties of CB_1 receptor blockade (Compton et al., 1996), as struggling is a more vigorous and active behavior than swimming and thus increased motor activity may shunt the behavioral response to a more active form. Qualitatively, animals treated with this regimen demonstrated a unique pattern of behavior consisting of long durations of struggling followed by prolonged periods of quiescence, which might explain why immobility was not reduced. 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 CB_1 receptor antagonist, has 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 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 endocannabinoid signaling could elicit a biphasic effect on depressive-like behaviors in the same manner as it does for anxiety-like behavior. For example, in tests of anxiety both CB_1 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 endocannabinoid 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 allow the conclusion that enhanced endocannabinoid activity occurs in almost all regimens that elicit an antidepressant response. Both sleep deprivation and environmental enrichment (Porsolt et al., 1978) 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 catabolism (Mechoulam et al., 1997), suggesting that the reduction in immobility in the FST seen following these regimens may in part be due to enhanced endocannabinoid activity. This idea is substantiated by the present data demonstrating that oleamide elicits a CB_1 receptor dependent reduction in immobility in the FST. The results of this study demonstrate that enhancement of endocannabinoid activity alone is sufficient to elicit an antidepressant response. This hypothesis is further supported by 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 endocannabinoid 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, are known to take a period of up to 2-6 weeks to become therapeutically effective (Thase et al., 2001). Pharmacological agents which influence endocannabinoid 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 endocannabinoid 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), the principal conclusion of this research is that inhibition of endocannabinoid 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 and anxiety (Gold and Chrousos, 2002).

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Figure Captions

Figure 1: The effect of AM404 administration on the occurrence of a) immobility; b) swimming behavior; and c) struggling behavior in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.

Figure 2: The effect of AM 251 pretreatment on the behavioral responses to AM 404 administration on the occurrence of a) immobility; and b) swimming behavior in the FST. Data are presented as mean time +/- SEM. All differences that were significant at $p < .05$ are denoted by *.

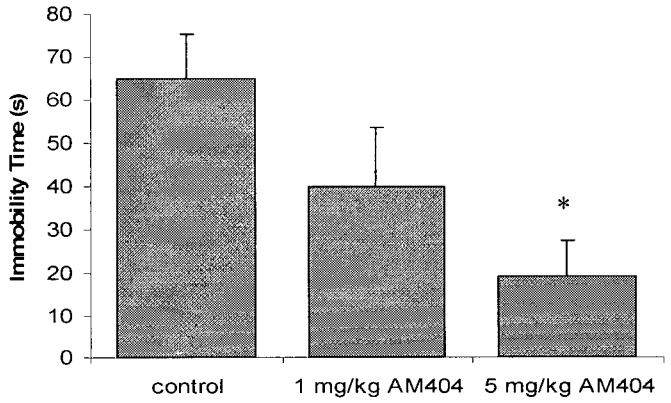
Figure 3: The effect of oleamide (OLE) administration on the occurrence of a) immobility; b) swimming behavior; and c) struggling behavior in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.

Figure 4: The effect of AM 251 pretreatment on the anti-immobility effect of oleamide (OLE) administration in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.

Figure 5: The effect of AM 251 administration on the occurrence of a) immobility; b) swimming behavior; and c) struggling behavior in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.

Figure 6: The effect of HU-210 administration on the occurrence of a) immobility; b) swimming behavior; and c) struggling behavior in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.

a)



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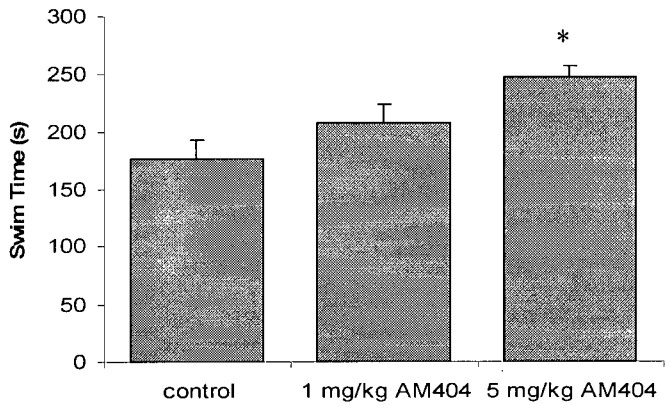

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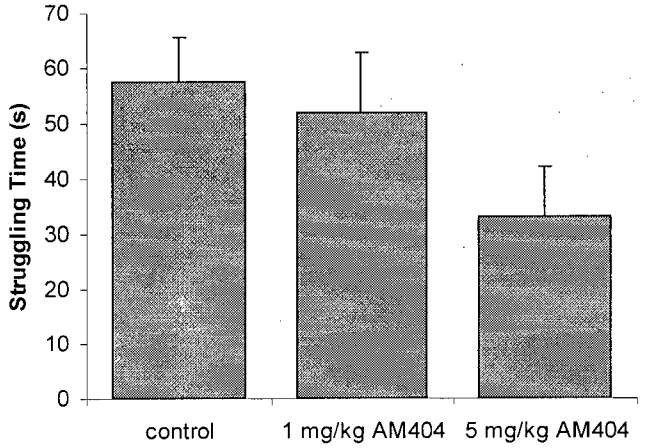
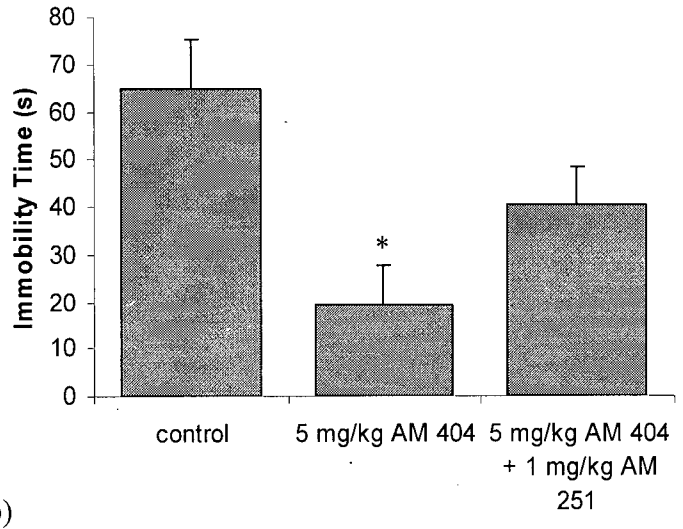


Figure 1: The effect of AM404 administration on the occurrence of a) immobility; b) swimming behavior; and c) struggling behavior in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.

a)



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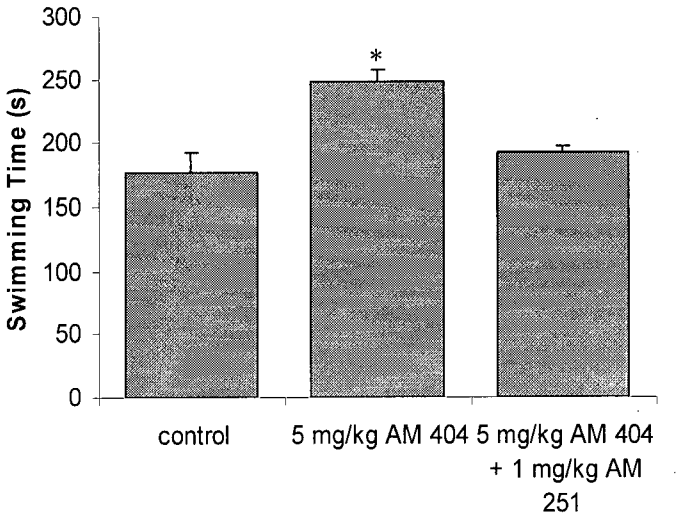
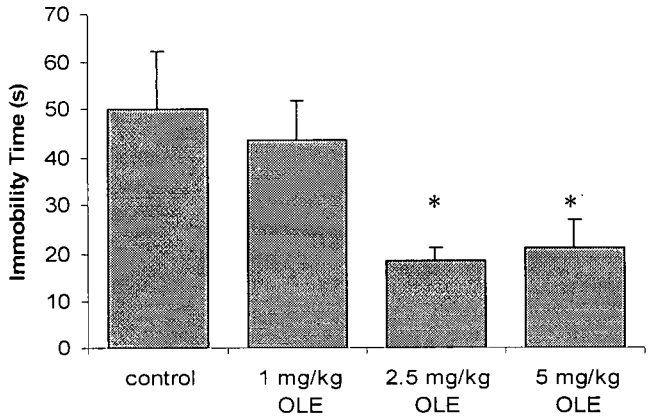


Figure 2: The effect of AM 251 pretreatment on the behavioral responses to AM 404 administration on the occurrence of a) immobility; and b) swimming behavior in the FST. Data are presented as mean time +/- SEM. All differences that were significant at $p < .05$ are denoted by *.

a)



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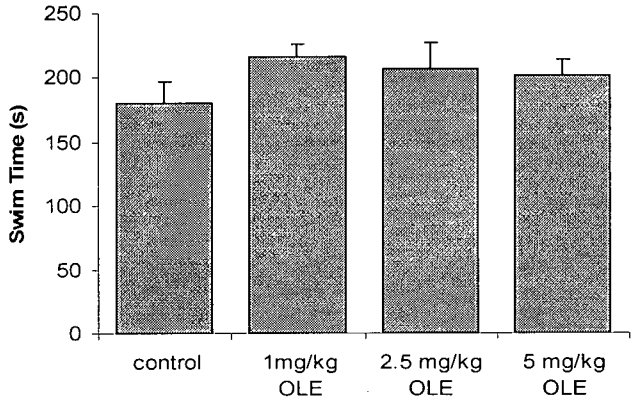

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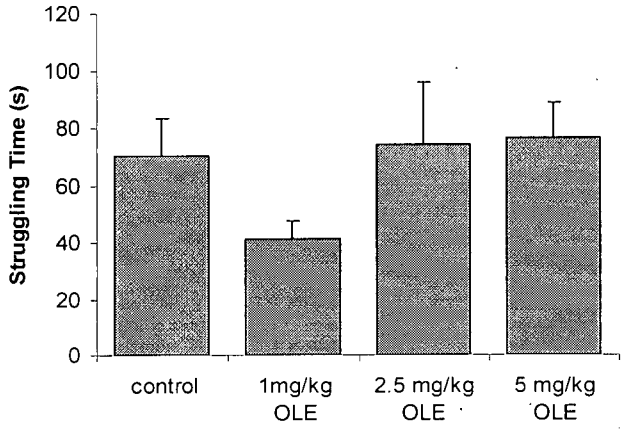

Figure 3: The effect of oleamide (OLE) administration on the occurrence of a) immobility; b) swimming behavior; and c) struggling behavior in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.

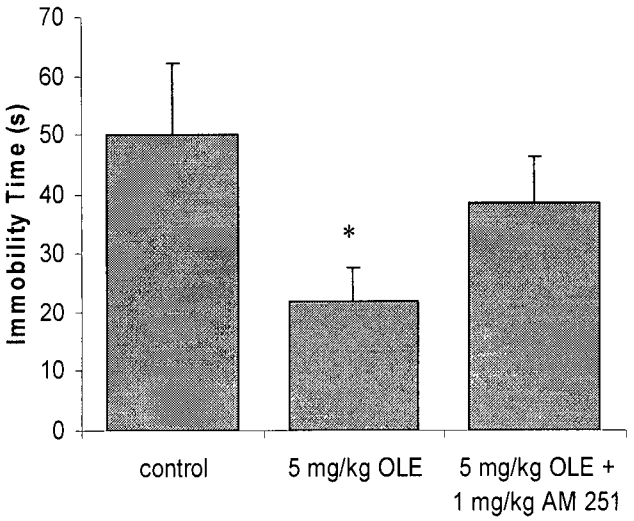

Figure 4: The effect of AM 251 pretreatment on the anti-immobility effect of oleamide (OLE) administration in the FST. Data are presented as mean time +/- SEM. All differences that were significant at $p < .05$ are denoted by *.

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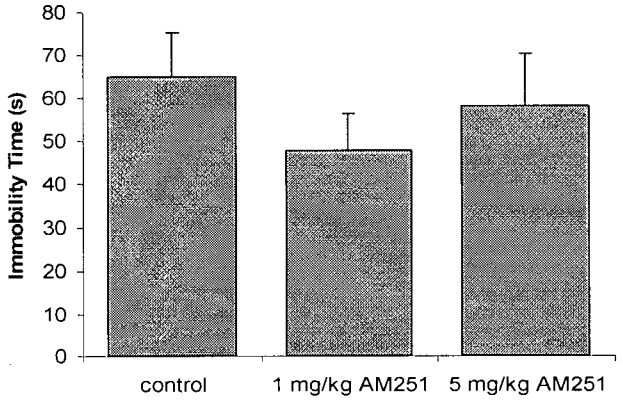

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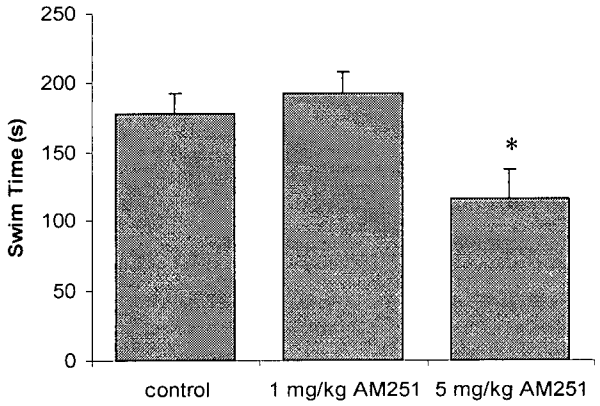

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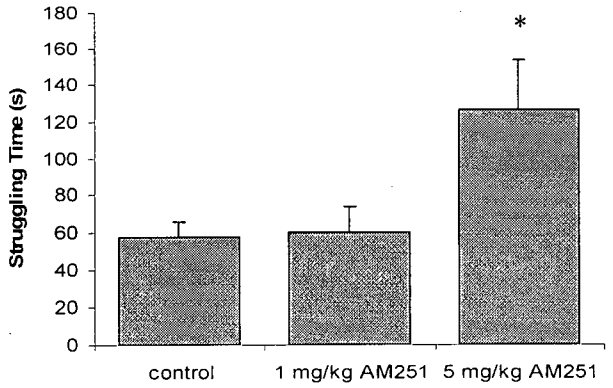
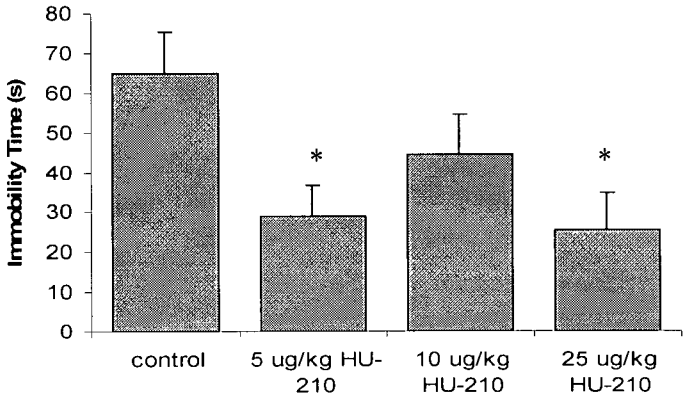


Figure 5: The effect of AM 251 administration on the occurrence of a) immobility; b) swimming behavior; and c) struggling behavior in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.

a)



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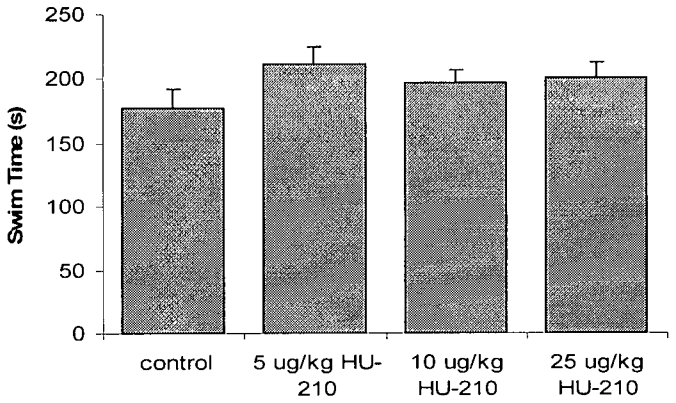

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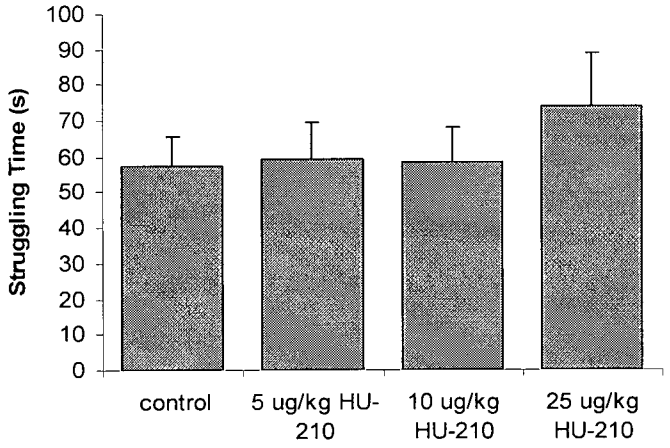

Figure 6: The effect of HU-210 administration on the occurrence of a) immobility; b) swimming behavior; and c) struggling behavior in the FST. Data are presented as mean time +/- SEM. All differences from the control group that were significant at $p < .05$ are denoted by *.