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Depression-like phenotype following chronic CB_1 receptor antagonism

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ABSTRACT

Rimonabant was the first clinically marketed cannabinoid $(CB)_1$ receptor antagonist developed to treat obesity. Unfortunately, CB_1 receptor antagonism produced adverse psychiatric events in patients. To determine whether this occurs pre-clinically, we investigated the effects of rimonabant in rodent models of mood disorders. Chronic treatment with rimonabant increased immobility time in the rat forced swim test and reduced the consumption of sucrose-sweetened water in an assay postulated to model anhedonia. These responses were similar to the effects elicited by chronic mild stress in these behavioral models, which, taken together, are indicative of a depression-like phenotype. Additionally, chronic treatment with rimonabant produced decreases in frontal cortex serotonin levels, marked reductions in hippocampal cell proliferation, survival, and BDNF levels, and elevations in the concentrations of pro-inflammatory cytokines including interferon gamma and TNF alpha. These preclinical findings mimic clinical reports and implicate possible mechanisms responsible for the unfavorable psychiatric events reported following chronic rimonabant use.

Introduction

In the past 30 years, the prevalence of obesity across America has doubled, with conservative forecasts estimating that nearly one third of adults in the United States are obese. Consequently, treatment strategies that successfully dampen the trajectory of these estimates should have substantial impact on numerous public health issues associated with obesity, including type 2 diabetes, cardiovascular disease, hypertension, and stroke (Brownell et al., 2009). The endogenous cannabinoid (CB) system governs, among other things, aspects of food intake and energy balance (Di Marzo and Matias, 2005), making it a viable target to curb appetite and to help reduce health concerns related to obesity. Several molecules targeting the CB system have been described, with the selective CB_1 receptor antagonist/inverse agonist, rimonabant, exemplifying the most advanced and well-characterized compound to date (Janero and Makriyannis, 2009).

Rimonabant possesses robust anti-obesity properties in patients (Padwal and Majumdar, 2007); however, its efficacy at reducing weight was paralleled by the development of adverse psychiatric disturbances including depressed mood disorders, anxiety, and suicidal ideation following long-term treatment (Christensen et al., 2007). A meta-analysis of the four pivotal clinical studies evaluating the anti-obesity effects of rimonabant revealed that these psychiatric complications were 2.5 times more likely to occur in patients receiving rimonabant (Mitchell and Morris, 2007) and the primary reason rimonabant's clinical use was discontinued. Moreover, chronic rimonabant administration was found to significantly increase anxiety responses measured on the Hospital Anxiety and Depression Scale (reviewed by Mitchell and Morris, 2007). While these clinical reports have ignited considerable debate (McPartland, 2009), behavioral studies from preclinical models have yielded equivocal results, with some reports indicating that rimonabant is anxiogenic (Navarro et al., 1997), while others suggest that the drug produces antidepressant- and anxiolytic-like actions (Griebel et al., 2005) or lacks antidepressant-like activity all together (Adamczyk et al., 2008) following acute dosing.

These clinical signs, which offer a unique opportunity to employ "reverse translational medicine,"–or moving from the clinic back to preclinical models–prompted us to explore the link between CB_1 receptors and behaviors associated with mood disorders and to investigate the possible molecular mechanisms responsible for the clinical side-effects of rimonabant. Based on the reported signs of depression and anxiety in patients treated chronically with CB_1 receptor antagonists, we hypothesized that long-term treatment with rimonabant would be accompanied by a depression-like phenotype in two behavioral models of depression: the forced swim test (FST) and rat sucrose preference test. Moreover, several mechanisms have been previously associated with depression-related

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behaviors including: (1) disruptions in cortical levels of biogenic amines such as serotonin (2) alterations in hippocampal cell proliferation, survival, and levels of brain-derived neurotrophic factor (BDNF) and (3) changes in the levels of cytokines throughout the central nervous system (CNS). Interplay between the serotonergic and CB systems has been described, with serotonin causing release of endocannabinoids from neurons (Best and Regehr, 2008), and conversely, endocannabinoids increasing serotonin (for review, Bambico et al., 2009). Likewise, a link between the endocannabinoid system, BDNF and neurogenesis has been established with BDNF regulating neuronal sensitivity to endocannabinoids (Maison et al., 2009) and exogenous CB_1 agonists triggering neurogenesis (Marchalant et al., 2009). Finally, the anti-inflammatory actions of CB1 agonists are well known (Marchalant et al., 2009; Buchweitz et al., 2008). Despite this evidence and rationale, the effects of chronic antagonism of CB_1 receptors on these mediators postulated to contribute to depression are unknown. We, therefore, hypothesized that chronic rimonabant administration may affect any or all of these potential mechanisms.

Materials and methods

Animals and drugs

All animal procedures were performed according to the guidelines of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* (Pub. 85-23, rev 1996). Male Sprague–Dawley rats (125–350 g, Harlan; Indianapolis, IN) were housed 2–3 per cage in an AAALAC-accredited facility and maintained on a 12-h light/dark cycle. The CB_1 antagonist rimonabant (Toronto Research Chemicals, Ontario, Canada) was suspended in 2% Tween 80/0.5% methylcellulose in water. Rimonabant or vehicle was administered intraperitoneally (i.p.) once daily for 21 (behavioral and microdialysis experiments) or 28 (cell proliferation, survival, BDNF and cytokine studies) days. Our choice of dose and route was based on previously published studies.

Chronic mild stress (CMS)

The CMS procedure was adapted from that of Grippo and colleagues (Grippo et al., 2003) and involved random exposure to a variety of mild stressors. A subset of animals (sham group) was housed in a separate room and served as a control group since this group of rats was not exposed to any CMS conditions. Sham animals were left undisturbed in their home cages, except once a week, when they were water deprived for 18 h followed by a 1-h sucrose consumption test.

Forced swim test (FST)

The FST consisted of a Plexiglas cylinder (45 × 20 cm, water depth 30 cm) filled with water (25 ± 1 °C). Following chronic treatment with vehicle, rimonabant (3–10 mg/kg, i.p.), CMS, or sham, animals were individually placed in the FST chamber and their behavior recorded by a video camera (Panasonic wv-BP140) connected to a video tracking system (Noldus Ethovision, Leesburg, VA). This tracking system was used to determine mobility (active movements) versus immobility behaviors during the 15-min swim test. Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by least squared differences (LSD) post-hoc analysis (SAS Institute, Cary, NC).

Sucrose consumption test

All animals were initially exposed to a bottle of 1% sucrose solution, followed by a baseline sucrose preference test that was conducted prior to the commencement of all drug or CMS protocols. The baseline tests, and all subsequent fluid intake tests, were conducted in animals deprived of water for 18–21 h. Water and 1% sucrose bottles were placed on the animal's home cage where they were allowed to consume the fluids for a period of 1 h. Fluid intake tests were performed once a week for a period of 3 weeks. Statistical significance was determined using a one-way ANOVA followed by least squared differences (LSD) post-hoc analysis (SAS Institute, Cary, NC).

In vivo microdialysis

Following chronic treatment with vehicle or rimonabant (10 mg/kg, i.p.), rats were anesthetized with isoflurane and a microdialysis guide cannula (CMA/12; CMA Microdialysis, Sweden) was positioned above the medial prefrontal cortex (mPFC; AP +3.2 mm; ML + 0.5 mm; DV −1.8 mm relative to bregma and dura) according to the stereotaxic brain atlas of Paxinos and Watson (Paxinos and Watson, 2007). Twenty-four hours after surgery, microdialysis probes (CMA Microdialysis, Sweden), with a 4-mm active membrane, were perfused with artificial cerebrospinal fluid at 1 μL/min and inserted into the mPFC. After a 3-h equilibration time, 4 samples (30 μl) were collected every 30 min and were analyzed for concentrations of serotonin (5-HT), norepinephrine and dopamine by HPLC (C18 ODS3 column, 150 × 3.0 mm, Metachem, Torrance, CA) with electrochemical detection (ANTEC, Netherlands).

Flow cytometry analysis of hippocampal cell proliferation and survival

For proliferation studies, rats were treated chronically with vehicle or rimonabant (3 or 10 mg/kg, i.p.) for 28 days and received BrdU injections (100 mg/kg, i.p., 4× per day, 2 h apart) prior to being sacrificed 24 h later. A similar treatment paradigm was used for survival studies, with the exceptions that prior to the start of repeated rimonabant treatment, rats received BrdU injections (100 mg/kg, i.p., 4× per day, 2 h apart) and then were sacrificed 21 days after the last injection of rimonabant. After treatment, hippocampal lobes were removed and the resultant cells were stained using the FITC BrdU Flow Kit (BD Biosciences San Jose, CA) as previously described (Balu et al., 2009).

BDNF measurements using quantitative real-time PCR (qPCR)

RNA was isolated from dissected brain regions using the RNeasy Fibrous Tissue mini Kit (Qiagen, Valencia, CA), and cDNA synthesis was performed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. qPCR reactions were carried out in an ABI PRISM 7900 Fast Real Time PCR System with Fast 96-well module (Applied Biosytems, Foster City, CA). The TaqMan gene expression ID for BDNF was Rn01484924_m1. Results were expressed as normalized to GAPDH mRNA expression. The effect of rimonabant treatment was determined by Student's *t*-test (one-tailed).

Cytokine measurements

The frontal cortex, hippocampus, or cerebellum was removed and homogenized in 400 μL buffer (150 mM NaCl, 20 mM Tris pH 7.5, 1 mM EDTA, 1 mM EGTA, 1% Triton-X-100, 0.1% SDS) containing complete protease inhibitor cocktail tablets (Roche, Indianapolis, IN). Electrochemiluminescence cytokine assays were performed on supernatants using capture antibody pre-coated 96-well multi-spot plates (MesoScale Discovery, Gaithersburg, MD) according to the manufacturer's instructions. The Rat Demonstration plate, consisting of multiplexed assays for GRO/KC, IFNγ, IL-1β, IL-4, IL-5, IL-13, and TNFα, was used in combination with detection antibodies labeled with the MSD SULFOTAG reagent. Cytokine concentrations were calculated using Prism (GraphPad Software, LA Jolla, CA). The

effect of rimonabant treatment was determined by Student's *t*-test (one-tailed).

Results

Chronic treatment with rimonabant produces a "depression-like" phenotype

Rimonabant (0, 3, or 10 mg/kg, i.p.) was administered to rats once daily for 21 consecutive days. This treatment paradigm was sufficient to produce a significant (5%) reduction in rat body weight at the dose of 10 mg/kg ($F_{(2,33)} = 7.08$; $p = 0.0028$), an effect that is consistent with the anti-obesity activity reported in clinical studies using this CB_1 receptor antagonist/inverse agonist (Padwal and Majumdar, 2007). In an automated version of the rat FST, chronic treatment with rimonabant (10 mg/kg) caused a significant ($F_{(3,42)} = 3.84$; $p = 0.0162$) and dose-dependent increase in immobility time that was similar to the effects of CMS ($p = 0.0027$) in this model (Fig. 1A). Furthermore, repeated rimonabant administration ($p = 0.0301$) or CMS treatment ($p = 0.0304$) resulted in a decrease in the consumption of sucrose-sweetened water (Fig. 1B). While no significant effect on total fluid intake was observed (Fig. 1B, inset; $F_{(3,46)} = 1.18$; $p = 0.3270$), rats treated chronically with rimonabant showed a trend towards decreasing total fluid consumption, which likely reflects the significant loss of weight observed in this treatment group. Based on reports that treatment with conventional antidepressants, including SSRIs (e.g., fluoxetine), results in a significant decrease in immobility time in the rodent FST and reverses CMS-induced decreases in sucrose intake (Rosenzweig-Lipson et al., 2007b; Papp and Sanchez, 2002), the responses observed in the present study are consistent with a "depression-like" phenotype in these preclinical assays.

Possible mechanisms underlying the "depression-like" behavioral phenotype: chronic rimonabant decreases frontal cortex levels of serotonin

The importance of serotonin in the pathophysiology of depression has been well documented since the late 1950s, with studies showing that lowering of CNS serotonin levels is sufficient to evoke depressive symptoms (reviewed by (Rosenzweig-Lipson et al., 2007a). *In vivo* microdialysis techniques coupled to HPLC-ECD in the rat mPFC revealed that 3-week rimonabant treatment (10 mg/kg, i.p., once per day) significantly ($F_{(1,16)} = 7.048$; $p = 0.0180$) reduced serotonin concentrations relative to rats treated chronically with vehicle (Fig. 2, left panel). It is worth noting that basal levels of serotonin recovered in the present studies are higher than previous reports using similar techniques in the same brain region. These differences likely reflect differences in chronic handling/dosing compared to acute treatment paradigms used in previous studies. Conversely, in the same animals, cortical levels of norepinephrine ($F_{(1,18)} = 1.357$; $p = 0.2601$) and dopamine ($F_{(1,15)} = 0.062$; $p = 0.8074$) were unaffected by long-term treatment with rimonabant (Fig. 2, middle and right panel, respectively).

Chronic rimonabant decreases hippocampal cell proliferation, survival, and BDNF levels

To explore additional mechanisms responsible for the behavioral phenotype of chronic CB_1 receptor antagonism, we studied the effects of chronic (28 days) rimonabant treatment on hippocampal cell proliferation and survival, as well as the effects on BDNF levels in the rat hippocampus. Flow cytometric analysis of BrdU incorporation in the rat brain revealed that treatment with rimonabant (0, 3, or 10 mg/kg, i.p.) produced a 25% and 20% reduction in hippocampal cell proliferation (Fig. 3A; $p = 0.031$) and survival (Fig. 3B; $p = 0.001$), respectively. Additionally, we found that long-term treatment with rimonabant significantly decreased cortical ($p = 0.0295$) and hippocampal ($p = 0.0122$) levels of the brain-derived neurotrophic factor (BDNF; Fig. 3C).

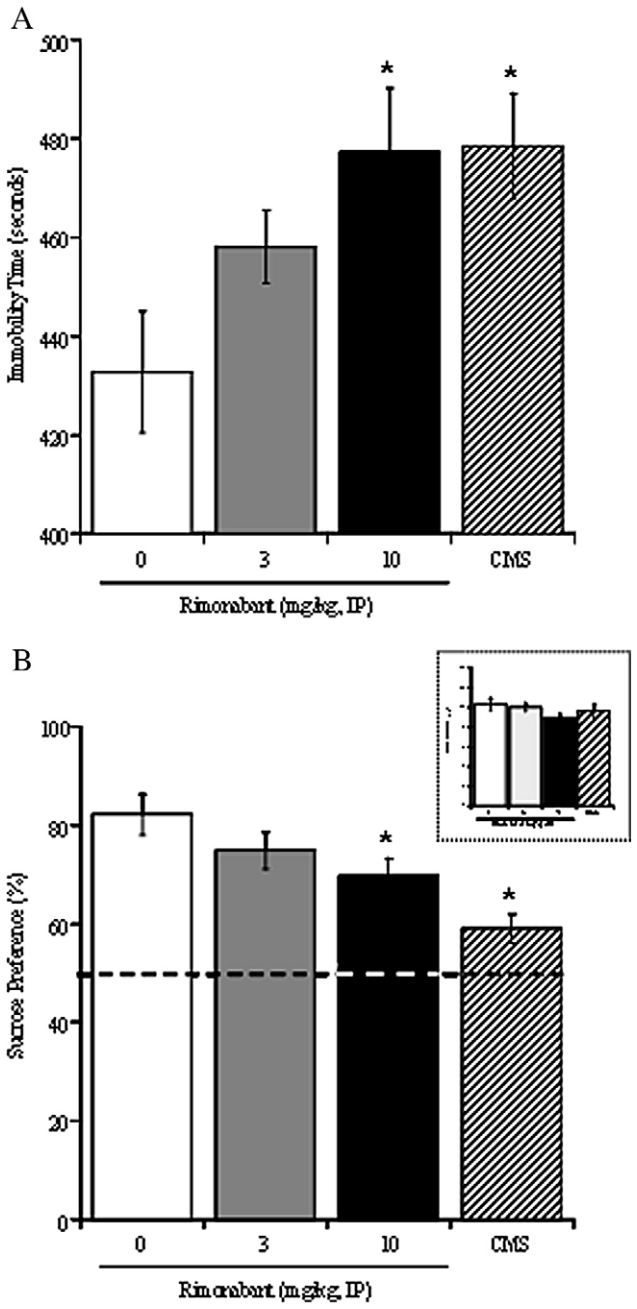


Fig. 1. Chronic treatment with rimonabant produces a depression-like phenotype in preclinical behavioral models of mood disorders. (A) In the rat forced swim test (FST), 21-day treatment with rimonabant (0–10 mg/kg, i.p., once per day) elicited a dose-dependent and significant increase in immobility time that was comparable to the effects elicited by chronic mild stress (CMS) over the same time period. Values are means ± SEM of seconds spent immobile during a 15-min swim test. $n = 11$–12 rats per treatment group. (B) In the sucrose preference test, vehicle-treated rats preferred sucrose-sweetened water more than 80% of the time. Conversely, rats treated chronically with either rimonabant or CMS consumed significantly less sucrose, resulting in responses that approached that of no preference (or 50%, designated by the dotted line). Values are means ± SEM of the percentage of time spent drinking sucrose-sweetened water. * Represents statistical ($p < 0.05$) differences from vehicle (for rimonabant) or sham (for CMS) treated animals. B (inset), Effects of both rimonabant and CMS were specific to sucrose preference as neither treatment significantly altered total fluid intake.

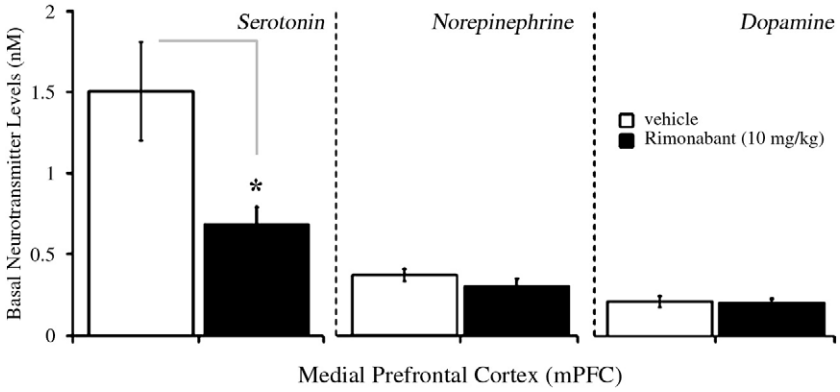


Fig. 2. Chronic treatment with rimonabant lowers frontal cortex levels of serotonin. Using *in vivo* microdialysis techniques in the rat mPFC, 3 week treatment with rimonabant (10 mg/kg, i.p., once per day) significantly and preferentially reduced serotonin concentrations relative to rats treated chronically with vehicle (left panel). Conversely, in the same animals, cortical levels of norepinephrine and dopamine were unaffected by chronic rimonabant treatment (middle and right panel, respectively). Values are means ± SEM of neurotransmitter concentrations (nM) collected every 30 min for 2 hours. * Represents statistical ($p<0.05$) differences from vehicle-treated animals.

Chronic rimonabant elevates cytokine levels in the frontal cortex and hippocampus

The role of pro-inflammatory cytokines in clinical depressive disorders (Himmerich et al., 2009) and rodent disease models (Dunn et al., 2005) has been well described. Consistent with these hypotheses, we found that the depression-like behavioral phenotype induced by repeated rimonabant administration was paralleled by a concomitant elevation in several pro-inflammatory cytokines. Repeated (28 days) treatment with rimonabant (10 mg/kg, i.p., once per day) produced a significant ($p=0.0189$) elevation in interferon gamma in the rat frontal cortex (Fig. 4A); however, hippocampal levels of this cytokine were not altered using this treatment paradigm ($p=0.2957$; Fig. 4A, right panel). Furthermore, chronic rimonabant elicited a significant increase in levels of tumor necrosis factor (TNF) alpha in the hippocampus ($p=0.0329$; Fig. 4B), produced a trend ($p=0.082$) to elevate concentrations of interleukin 4 (IL-4) in the hippocampus (Fig. 4C), but did not affect levels of the neutrophil chemokine, GRO/KC, in any brain region tested (Fig. 4D).

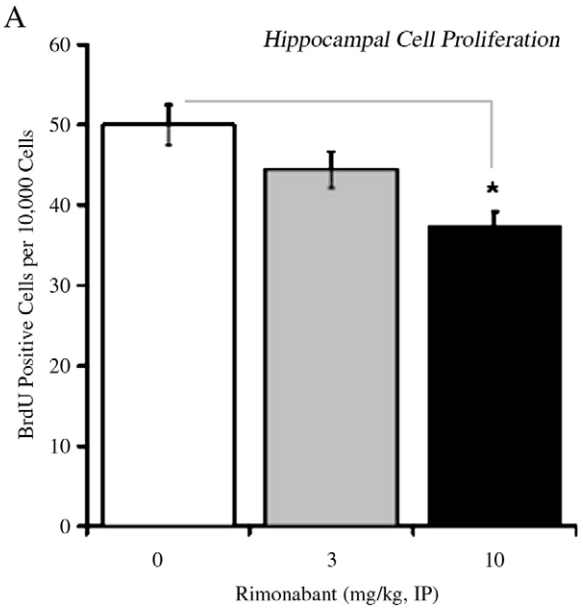
Discussion

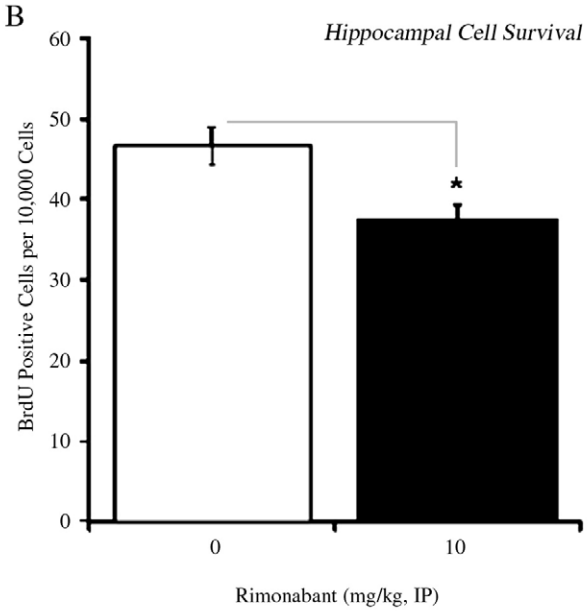
Over the past two decades, several pharmacological treatment options for obesity have been proposed (Cooke and Bloom, 2006), with the most recent of these being rimonabant (Acomplia®), a selective CB_1 receptor antagonist/inverse agonist. Although rimonabant exhibited efficacy at reducing weight in clinical settings (Padwal and Majumdar, 2007), its long-term use was associated with the development of adverse psychiatric events (Christensen et al., 2007; Mitchell and Morris, 2007). We took a "reverse translational medicine" approach to investigate the preclinical phenotype induced by repeated rimonabant treatment and to establish a mechanistic link between CB_1 receptor antagonism and mood disorders. The present experiments provide direct evidence that chronic treatment with rimonabant produces *preclinical* behaviors that are consistent with a "depression-like" phenotype. Specifically, 3-week administration of rimonabant, at a dose that significantly reduced rodent body weight, produced an increase in immobility time in the rat FST as well as reduced the consumption of sucrose-sweetened water in an assay proposed to be a preclinical model of anhedonia – a core symptom domain of major depressive disorders (Moreau, 1997). These results bring preclinical findings into alignment with clinical observations and afford the opportunity to explore the molecular mechanisms responsible for the psychiatric disturbances produced by persistent antagonism of the CB_1 receptor.

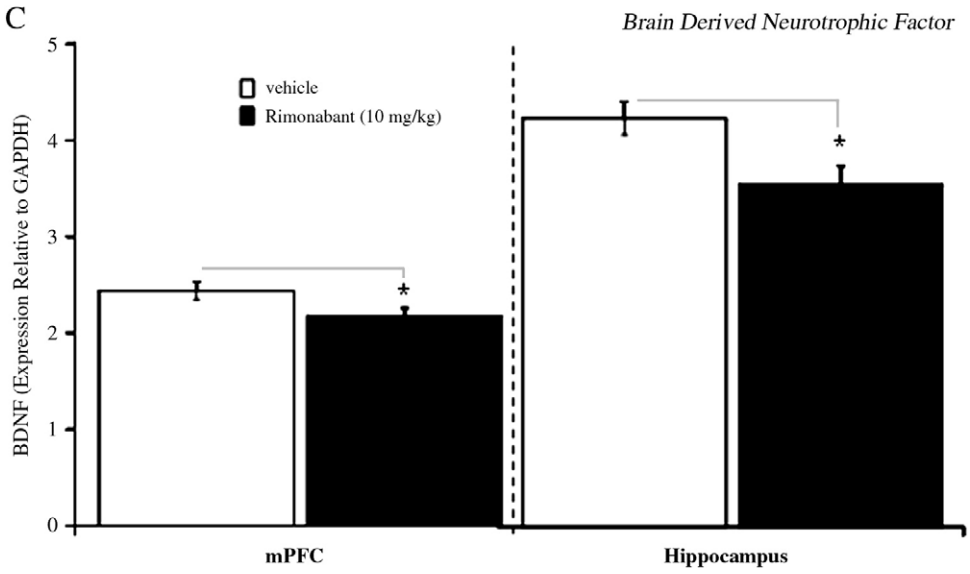
Clinical and preclinical evidence converge on a common hypothesis that the endogenous CB receptor system mediates facets of mood disorders (Bambico and Gobbi, 2008). In fact, the weight loss caused by CB_1 antagonism may, at least in part, be explained by effects on the dopaminergic system, such that antagonism inhibits dopamine release in response to palatable food in rats (Melis et al, 2007), providing a clear link between the effects of CBs on mood and food intake. Furthermore, activation of CB_1 receptors by a variety of agonists elicits antidepressant- and anxiolytic-like responses in several different behavioral disease models (Bambico et al., 2007), while pharmacological antagonism (Navarro et al., 1997) or genetic deletion of CB_1 receptors (Martin et al., 2002) results in the opposite (pro-depressive) effects. Our findings support this hypothesis insomuch that the behavioral responses to chronic rimonabant treatment in the rodent FST and sucrose preference tests mirror the effects elicited by CMS (present study) as well as with the effects reported by others using chronic stress paradigms (Papp and Sanchez, 2002). Interestingly, while CMS was used as a positive control in our behavioral experiments, previous reports indicate that CMS may or may not affect body weight (Bekris et al., 2005; Grippo et al., 2006), respectively). Overall, the behavioral responses observed in these assays are consistent with a depressive-like phenotype, an argument supported by the fact that antidepressants decrease immobility time in the FST and reverse stress-induced decreases in sucrose consumption. As the behavioral measurements used in the current study are frequently employed in the field, our findings support the proposal that chronic rimonabant treatment represents a unique, pharmacological-induced model of depression; however, final confirmation of this hypothesis awaits additional empirical validation.

Having established that chronic rimonabant treatment results in a depression-like phenotype *in vivo*, we were interested to investigate the molecular mechanisms underlying these behavioral observations. As loss of monoaminergic tone (Rosenzweig-Lipson et al., 2007a), down-regulation of growth factors and subsequent reduction in cell proliferation and survival (Malberg and Schechter, 2005), and increased production of pro-inflammatory cytokines (Dunn et al., 2005) are among the principle mechanisms implicated in depression in the clinic, we initially focused our investigation around these possible molecular changes. First, we found that chronic rimonabant treatment elicited a significant decrease in frontal cortex serotonin levels, an effect consistent with the monoamine hypothesis of depression–which postulates that the debilitating and chronic symptoms of depression result from the dampening of central levels of serotonin–as well as with the proposed mechanism of action of antidepressant drugs (Rosenzweig-Lipson et al., 2007a).

Second, we revealed that the behavioral phenotype induced by chronic rimonabant was accompanied by a marked reduction in hippocampal cell proliferation and survival. This is consistent with a number of recent findings supporting a firm link between depression-







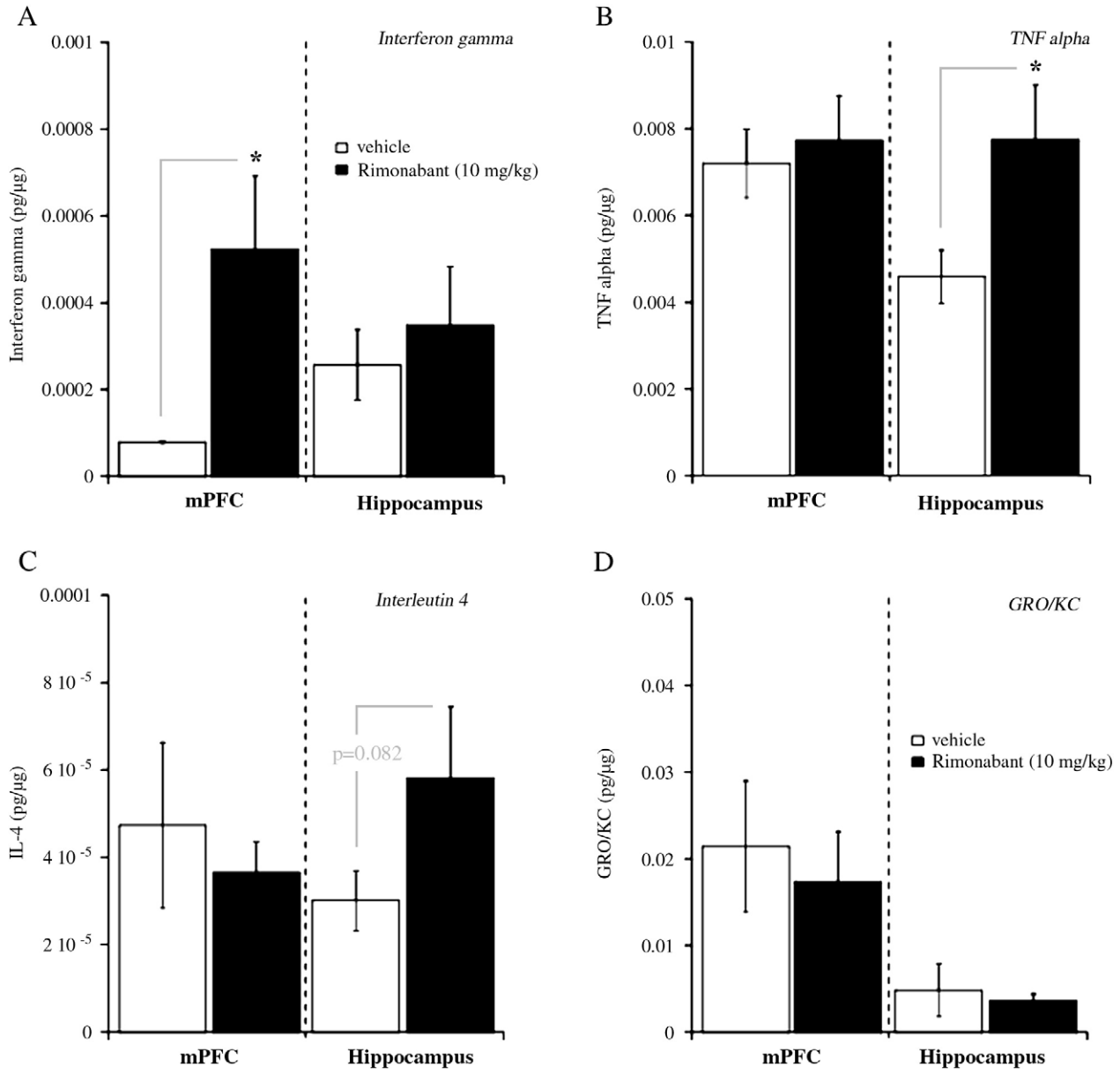


Fig. 4. Chronic treatment with rimonabant elevates cytokine levels in the CNS. (A) Consistent with the hypothesis that pro-inflammatory cytokines play a role in the pathophysiology of mood disorders, repeated (28 days) treatment with rimonabant (10 mg/kg, i.p., once per day) produced a significant ($p = 0.0189$) elevation in interferon gamma in the rat frontal cortex, without impacting levels of this cytokine in the hippocampus (left and right panel, respectively). (B) In addition, chronic rimonabant administration resulted in a significant increase in levels of TNF alpha in the hippocampus (right panel), produced a trend ($p = 0.082$) to elevate concentrations of IL-4 in the hippocampus (C, right panel), and did not alter levels of the neutrophil chemokine, GRO/KC, in any brain region tested (D). Values are means ± SEM of cytokine/chemokine concentrations (pg/µg). * Represents statistical ($p < 0.05$) differences from vehicle-treated animals.

related behaviors and altered cell proliferation and cell survival (or neurogenesis) in the hippocampus (Malberg and Schechter, 2005). This is also consistent with the observation that CMS also decreases survival of hippocampal new born cells (Lee et al., 2006); however, in contrast to the effects of chronic rimonabant administration, CMS did not significantly impact hippocampal cell proliferation. Although we did not confirm the neuronal nature of the cells in our study, these findings are in line with what is found following various physical and psychosocial stressors and are the opposite of what is produced following chronic treatment with chemical antidepressants (Malberg and Schechter, 2005). A likely contributing factor to decreased cell proliferation and survival produced by long-term treatment with rimonabant was the significant and coincident reduction in BDNF expression in both the hippocampus and frontal cortex. These findings, taken in context with our behavioral data, are remarkably consistent with several recent studies demonstrating a link between

Fig. 3. Chronic treatment with rimonabant decreases cell proliferation and survival in the rat hippocampus. The effects of rimonabant treatment on hippocampal cell proliferation and survival were studies using two different treatment protocols. For proliferation studies, rats were treated chronically with rimonabant (0–10 mg/kg, i.p., once per day) for 28 days and received BrdU injections (100 mg/kg, i.p., 4× per day, 2 h apart) prior to being sacrificed 24 h later. For survival studies, rats received BrdU injections (same injection procedures reported for proliferation studies) 1-day prior to the start of chronic rimonabant (10 mg/kg, i.p.) or vehicle treatment. All rats were then sacrificed 21 days after the last drug or vehicle injection. (A) Hippocampal cell proliferation and (B) survival, as measured by a flow cytometry method recently described by Balu and colleagues (2009), was found to be significantly decreased following chronic treatment with rimonabant. Values are means ± SEM of BrdU positive cells/10,000 cells. (C) Chronic (28 days) treatment with rimonabant also resulted in a significant decrease in hippocampal and frontal cortex BDNF mRNA expression. Values are means ± SEM of BDNF expression relative to GAPDH. * Represents statistical ($p < 0.05$) differences from vehicle treated animals.

BDNF signaling, neurogenesis, and behavior. In particular, knockdown of BDNF in subregions of the hippocampus (Taliaz et al., 2009) and ablation of the BDNF receptor (TrkB) is paralleled by a hastening of depression-like behaviors (Li et al., 2008).

Third, we determined that chronic rimonabant treatment increased CNS levels of a number of pro-inflammatory cytokines. Specifically, rimonabant significantly elevated levels of interferon gamma in the rat mPFC and TNF alpha levels in the hippocampus, while producing a trend towards increased IL-4 in the rat hippocampus. The link between incidences of depression and interferon gamma treatment have been described (Dunn et al., 2005), and our findings provide additional support for the role of pro-inflammatory cytokines in the pathophysiology of mood disorders. Moreover, as cytokine-induced sickness behaviors resemble symptoms of depression, and mood disorders are often co-morbid with other diseases, some of which–pain, for example–have an inflammatory component and thus elevated cytokine levels (Stein et al., 2009), it can be argued that altered cytokine expression is a contributing factor to the behavioral effects of chronic rimonabant treatment.

Overall, these data demonstrate that chronic rimonabant treatment induces a depression-like behavioral phenotype in rodents. Furthermore, we show that these behavioral responses are accompanied by marked decreases in frontal cortex serotonin concentrations, reductions in hippocampal cell proliferation, survival, and BDNF expression, and significant elevations in central levels of several pro-inflammatory cytokines including interferon gamma, TNF alpha, and IL-4. Interestingly, while CMS was used as a positive control in our behavioral experiments, previous studies have shown that it causes a decrease in serotonergic activity in the prefrontal cortex (Bekris et al., 2005) as well as increase production of several pro-inflammatory cytokines such as TNF alpha and IL-1 beta (Grippo et al., 2005). These findings along with our own suggest that these changes may be central to a depressive-like phenotype. Our intention in this short communication was to translate clinical findings around rimonabant-induced psychiatric events back to a preclinical setting, and in doing so, provide the behavioral and molecular basis for a novel and complex model of pharmacological-induced depression that reflects the human condition.

Considering the pharmacokinetic/pharmacodynamic translation between humans and rats, the dose used in the current study produced a reduction in body weight, indicating a pharmacodynamic translatability and while limited pharmacokinetic data is available for rimonabant (Patel and Pathak, 2007), comparison is warranted. In rodents, the dose that we choose (10 mg/kg) results in greater than 50% receptor occupancy over an 8 hour period (Rinaldi-Carmona et al., 1995) when dosed by a less efficient route (p.o.) or and when dosed i.p. (which is in-line with our studies). Furthermore, a 10-mg/kg dose of rimonabant resulted in plasma and brain concentrations in the ten to hundreds of ng/ml range (McCulloch et al., 2008; Ward et al., 2009, respectively). Clinically rimonabant has been dosed at 5, 20, 40 and 90 mg (Patel and Pathak, 2007; Huestis et al., 2007) and following 40 mg the mean maximum plasma concentration was 308 ng/ml. These studies, taken together, allow us to conclude that the expected exposures in our rodent studies are comparable to those achieved in the clinic.

In summary, in addition to presenting a novel preclinical model of depression, we observed a concurrence of three mechanisms previously linked to depression—evidence that provides additional compelling support for a central role of these mechanisms in clinical depression. Upon further empirical evaluation, including investigating the effect of standard antidepressant treatments such SSRIs, chronic rimonabant administration to rodents in combination with the behavioral consequences reported herein, may be utilized to scrutinize the efficacy of novel therapeutic interventions designed to alleviate mood disorders. Moreover, since rimonabant has already been shown to induce depressive symptoms in humans, chronic rimonabant administration to rats presents a novel way to model the etiology and pathological progression of mood disorders.

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