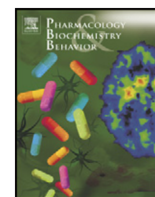
Pharmacology, Biochemistry and Behavior 100 (2012) 506–512

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh



The effects of chronic administration of tranylcypromine and rimonabant on behaviour and protein expression in brain regions of the rat

Neda Assareh *, Maha M. ElBatsh [1], Charles A. Marsden, David A. Kendall

School of Biomedical Sciences and Institute of Neuroscience, University of Nottingham, Nottingham NG7 2UH, UK

ARTICLE INFO

Article history:
Received 29 June 2011
Received in revised form 3 October 2011
Accepted 14 October 2011
Available online 20 October 2011

Keywords:
Brain-derived neurotrophic factor (BDNF)
Rimonabant
Depression
Endocannabinoids
Tranylcypromine (TCP)

ABSTRACT

Recent findings indicate that CB1 receptor blockade might be relevant to the action of antidepressant drugs as inhibition of endocannabinoid function can increase synaptic availability of neurotransmitters; an effect also seen with chronic antidepressant drug treatment. Chronic treatments with established antidepressants also lead to raised brain BDNF levels. The aim of this study was to compare the effects of rimonabant (an inverse agonist/antagonist of CB1 receptors) with those of the antidepressant tranylcypromine (TCP), on behaviour and expression of BDNF/CREB signalling pathways in rat brain.

Daily (i.p.) injections of vehicle or TCP (10 mg/kg) or rimonabant (2 mg/kg) were given for 14 days. Locomotor activity (LMA) and a conditional emotional response (CER) were measured in addition to levels of BDNF and CREB/phospho-CREB, using immunoblotting, in the frontal cortex, hippocampus, striatum and cerebellum. The velocity of movement was increased significantly on the 3rd, but not 9th, day of TCP treatment *versus* vehicle-treated rats ($p<0.05$) while rimonabant had no effect. There were no significant changes in grooming or freezing behaviours after rimonabant or TCP compared to vehicle-treated rats. Rearing was significantly reduced by TCP treatment on the 3rd, but not 9th, day of treatment ($p<0.001$) while rimonabant had no effect. BDNF levels were significantly increased in the frontal cortex after TCP ($p<0.05$) but not by rimonabant. Neither TCP nor rimonabant significantly affected CREB or p-CREB expression.

In conclusion, chronic administration of TCP to rats increased BDNF expression in the frontal cortex but no similar effect was observed with rimonabant indicating that rimonabant does not show antidepressant drug-like responses after chronic treatment.

© 2011 Elsevier Inc. All rights reserved.

1. Introduction

Endocannabinoids, such as anandamide and 2-arachidonoyl glycerol (2-AG), are believed to be synthesised “on demand” from membrane-located precursors in postsynaptic neurons (Baker et al., 2003) in response to increasing intracellular calcium levels. Anandamide and 2-AG bind to pre-synaptic CB1 receptors leading to inhibition of presynaptic Ca^{2+} influx and a decrease in the release of neurotransmitters from the presynaptic nerve endings. Several studies have demonstrated that this CB1 receptor signal transduction system, could impact on biochemical pathways that are relevant to depression, such as emotional processing and memories (2005, Wilson and Nicoll, 2002). In the clinic, depression is treated primarily using drugs that directly enhance the availability and release of brain monoamine neurotransmitters (Skolnick et al., 2001). Drugs acting on monoamines in the brain activate second messenger signal transduction pathways including cyclic AMP (cAMP), phosphatidylinositol hydrolysis and intracellular calcium, and consequently cause up-regulation of the cAMP response element binding protein (CREB) and the transcription factor phospho-CREB leading to altered expression of neuroprotective genes such as brain derived neurotrophic factor (BDNF; (Duman, 1998; Lesch, 2001)).

Altar (1999) demonstrated that chronic administration of selective serotonin reuptake inhibitors and electroconvulsive therapy (ECT) caused increased expression of neurotrophins indicating their involvement in the suggested neuroprotective effect of antidepressants. It has been suggested that CB1 receptor antagonists might contribute to improving depressive symptoms by increasing monoaminergic neurotransmission and thus be a novel pharmacotherapeutic approach to mood disorders (Witkin et al., 2005).

Dysregulation of the HPA axis has a key role in the neurobiology of depression (Hill and Gorzalka, 2005). Hyperactivity of the HPA axis and reduced feedback inhibition are observed in depressed humans and in animal models (Patel and Hillard, 2009). Additional evidence indicates that cannabinoid compounds also affect the HPA axis (Manji et al., 2001). Activation of the HPA axis can occur via activation

* Corresponding author at: 11/22 Evans Ave Eastlakes, Sydney NSW 2018, Australia. Tel.: +61 422881674; fax: +61 115 82 30142.

E-mail addresses: n_assareh@yahoo.com (N. Assareh), mahaelbatsh@yahoo.com (M.M. ElBatsh), charlesmarsden@hotmail.co.uk (C.A. Marsden), Dave.Kendall@nottingham.ac.uk (D.A. Kendall).

[1] Present address: Clinical Pharmacology Department, Faculty of Medicine, Menoufia University, Shebin Elkom, Egypt.

0091-3057/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2011.10.017

of corticotropin releasing factor (CRF) following CB1 receptor activation (Rodriguez de Fonseca et al., 1995). Anxiogenic effects of cannabinoid receptor agonists have been suggested to be a direct action of these compounds in the hypothalamus leading to activation of the pituitary–adrenal axis. Therefore, the effects of cannabinoid receptor agonists acting via CRF activation can be attenuated by CRF receptor antagonists (Rodriguez de Fonseca et al., 1996), compounds that may potentially act as anti-depressant drugs. Witkin et al. (2005) suggested that CB1 receptor antagonists could decrease mRNA expression of CRF (Gonzalez et al., 2004) and thus prevent the anxiogenic HPA response via activation by CB1 receptors. In support of this, rimonabant inhibits the elevation of corticotropin and corticosterone induced by Δ^9-THC (Griebel et al., 2005; Manzanares et al., 1999; Murphy et al., 1998), providing evidence of an antidepressant-like effect of the CB1 receptor antagonist in the control of CRF-HPA function.

There is also evidence indicating some antidepressant properties of CB1 receptor antagonists. For example, the novel CB1 antagonists SLV319 and AVE1625 increased dopamine and acetylcholine release in rat pre-frontal cortex (Witkin et al., 2005). It has also been reported that rimonabant increases noradrenaline and 5-HT levels in rat pre-frontal cortex and hippocampus and that antidepressant-like effects of acute rimonabant were observed in rodent behavioural models (Griebel et al., 2005). In contrast, it has been suggested that blockade of CB1 receptors by either pharmacological or genetic methods could induce states similar to melancholic depression (Hill and Gorzalka, 2005). Christensen et al. (2007) found that 20 mg/day of rimonabant increased the risk of psychiatric symptoms such as mood depression and anxiety. Furthermore the US Food and Drug Administration (FDA, 2007) reported that the risk of suicide was increased in individuals during clinical trials for obesity treatment with rimonabant; this lead to the removal of rimonabant from the market and continuing concern has focused on the possible precipitation of depressive symptoms in obese rimonabant-treated individuals (Pi-Sunyer et al., 2006). Although, acute administration of rimonabant has shown anti-depressant-like activity in animal models it is important that the effects are also investigated following chronic treatment.

The objective of this study was to determine whether chronic treatment with either rimonabant, (2 mg/kg/day) or the conventional antidepressant drug, tranylcypromine, (TCP) a monoamine oxidase inhibitor, (10 mg/kg/day) would produce similar physiological (body weight, food consumption), behavioural (conditional emotional response, locomotor activity) and protein expression (BDNF and CREB/phospho-CREB) changes in rats.

2. Materials and methods

2.1. Animals

24 male Lister hooded rats (150–200 g) were purchased from the Biomedical Sciences Unit, University of Nottingham (a colony derived from Charles River UK stock). Animals were randomly placed in 3 groups. They were injected with either TCP (10 mg/kg/day, i.p.) or rimonabant (2 mg/kg/day) or vehicle for 14 days between 9 and 10 am. All rats were kept under 12:12 h of light/dark cycle at a constant temperature of 21 ± 2 °C and 40–60% humidity with food and water available *ad libitum*. All experiments were performed under UK Home Office (1986) Animal Scientific Procedures Act and with local University of Nottingham Ethical committee approval (PPL 40/2715).

2.2. Drugs

TCP (Sigma, 10 mg/kg/day), rimonabant (National Institute of Mental Health, 2 mg/kg/day) and vehicle were prepared using the following formula: ethanol, Tween80, saline, 3:1:16 respectively. The drug solutions were injected in a volume of 1 mg/kg i.p. The dose of TCP was selected on the basis of other studies that reported (Nibuya et al., 1995, 1996) chronic effects of the drug on BDNF expression in the frontal cortex of rats. Rimonabant (2 mg/kg) used as this dose has been demonstrated to blocked the anxiolytic effects of raising endogenous cannabinoid tone by administration of FAAH inhibitors in the rat (Kathuria et al., 2003). Furthermore this dose has been shown to antagonise the effects of THC-evoked excitation of the ventral tegmental area neurons (French, 1997) and WIN55212-2 (a CB1 receptor agonist) induced stimulation of locus coeruleus neurons (Mendiguren and Pineda, 2006), Rimonabant (2 mg/kg) also selectively decreased the development of nicotine induced sensitivity (Kelsey and Calabro, 2008) while French (1997) concluded on the basis of behavioural and *ex vivo* binding studies that a dose of 0.5 or 2 mg/kg blocked CB1 receptors.

2.3. Physiological measurements

Body weight and food consumption (by subtraction of consumed food from the total amount provided) were measured daily between 9 and 10 am.

2.4. Behavioural tests

Locomotor activity (LMA); and conditional emotional behaviour response (CER) were assessed. On the second day of the experiment, animals were habituated to the locomotor activity boxes and LMA was then assessed on the 3rd and 9th days of treatment over 20 min in the same activity boxes. The locomotor activity test was performed using transparent Perspex boxes (40 × 20 × 25 cm) combined with computer tracking using Ethovision (Noldus, Wageningen, Netherlands). The animals were allowed to freely explore the arena. In the LMA trials a video camera was installed to record all behaviours either on tape for manual scoring of grooming (individual action of an animal to cleaning the body surface by licking, *etc.*) and rearing (standing straight on both hind legs) or directly to a monitor in order to calculate the total distance and velocity of movement with the Ethovision software.

CER; in order to assess contextual learning, unconditioned stimuli (UC) were applied to the rats in a metallic observation chamber (25 × 24 × 49 cm) by inducing electrical foot-shock. Animals received foot-shocks in this box on the 10th day of treatment (conditioning day), and CER was assessed on the 11th, day of the experiment. The floor of the chamber was covered with 21stainless steel rods set 1.1 cm apart and connected to a shock generator (Campden Instruments, Loughborough, UK). A video camera recorded all behaviours. On the conditioning day, rats were individually placed in the chamber and, after 30 s received foot-shocks (0.4 mA, for 1 s × 10 one/min for 10 min) and 30 s after the last shock the rats were returned to their home cage. On the trial day, rats were placed in the same chamber in which they had received foot shocks 24 h previously and the total duration of freezing behaviour (total absence of movement excluding breathing and heart beat) during 10 min was manually measured.

Rats were killed by decapitation on the day following the end of the treatment and their brains dissected on ice into regions (pre-frontal cortex, striatum, hippocampus, cerebellum) then immediately frozen at −80 °C. The frontal cortex, hippocampus, striatum and cerebellum were separated into cytosolic and nuclear fractions on the day of immunoblotting.

2.5. Protein expression

BDNF, CREB and p-CREB protein expression were measured in the frontal cortex, hippocampus, striatum and cerebellum using Western immunoblotting. Total protein in each sample was measured (Lowry et al., 1951) and ß-actin expression was used as a reference protein

to account for any differences in sample loading. BDNF expression was assessed in 50 μg samples using 15% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). CREB and p-CREB proteins were detected using 10% SDS-PAGE in samples of approximately 30 μg and 120 μg respectively. Blots were incubated with appropriate primary antibodies. The primary antibody used for BDNF was BDNF (N-20), a rabbit polyclonal IgG (Santa Cruz Biotechnology) at a dilution of 1:500 in 5% milk. The CREB antibody was CREB (48H2) a rabbit mAb (Cell Signalling Technology) (1/1000) and anti-phospho-CREB (Ser 133) was a rabbit polyclonal (Cell Signalling Technology) (1/500). The samples were incubated overnight at 4 °C and then BDNF, CREB and p-CREB protein were incubated with a secondary antibody, 1/2000 dilution in blocking buffer (5% milk), a polyclonal goat anti-rabbit conjugated with horse-radish peroxidase (HRP) (Dakocytomation, Denmark). For β-actin identification the secondary antibody was a polyclonal goat anti-mouse conjugated with Horse-Radish Peroxidase (Dako) at a dilution of 1/2000 in blocking buffer (5% milk), for 60 min incubation at room temperature. The blots were exposed to hyper ECL autoradiography film (Amersham Bioscience, UK) in the dark room. BDNF, β-actin, CREB and p-CREB blots were exposed for15 min, 30 s, 5 min and 60 min respectively. The predicted molecular weights of BDNF and β-actin bands were 14 kDa and 43 kDa, both CREB and p-CREB about 43 kDa. The developed films were scanned using a Gs-710 imaging Densitometer (Bio-Rad) using the Quantity One software programme (Bio-Rad, Biosciences) for image analysis. The proteins levels are presented as a percentage change from the vehicle-treated animals (defined as 100%).

2.6. Statistical analysis

Data are presented as means ± standard error of the mean (SEM). The data from the food consumption and body weight measurements were analysed using two-way ANOVA with Prism 4.0. The data from either LMA or CER tests (total distance movement, velocity, total rearing, total grooming and freezing) on the days of behavioural test and protein expression in the frontal cortex, hippocampus, striatum and cerebellum were analysed using one-way ANOVA followed by Dunnett's multiple comparison test when appropriate.

3. Results

3.1. Effects of chronic treatment with TCP or rimonabant on physiological measurements (gain in body weight, food consumption)

TCP (10 mg/kg i.p.) significantly decreased body weight compared with control, during 14 days of treatment (F (2, 294) = 3.74, p = 0.0408) while rimonabant (2 mg/kg) had no effect on body weight (Fig. 1). Chronic administration of TCP (10 mg/kg i.p.) significantly reduced (F (2, 39) = 13.55, p = 0.0315) food consumption compared to vehicle-treated controls while rimonabant (2 mg/kg) again had no effect (Fig. 2).

3.2. Effect of chronic treatment with TCP (10 mg/kg/day) or rimonabant (2 mg/kg/day) on locomotor activity and CER

Neither TCP (10 mg/kg/day) nor rimonabant (2 mg/kg/day) altered the total distance moved during the 20 min experimental period on either the 3rd or 9th day of treatment (data not shown). TCP significantly increased ($p<0.05$) the velocity of locomotion over 20 min, compared to vehicle on the 3rd day of treatment, an effect not observed in the rimonabant-treated rats (Fig. 3.A). Neither TCP nor rimonabant affected the velocity on the 9th (Fig. 3.B) day of treatment. TCP significantly reduced ($p<0.001$) total duration of rearing in TCP-treated rats compared with vehicle-treated controls on the 3rd day (Fig. 4.A) but there was no difference on the 9th day of treatment (Fig. 4.B). Total duration of rearing was unaffected by rimonabant on either the 3rd or 9th days of treatment (Fig. 4, A, B respectively). Total duration of grooming was unaffected by either TCP or rimonabant on both the 3rd and 9th days over the 20 min experimental period, although rimonabant tended to increase grooming on the 3rd day of the experiment (Fig. 5.A,B respectively). Neither TCP nor rimonabant had a significant effect on the total time spent freezing in the CER test (day 11, data not shown).

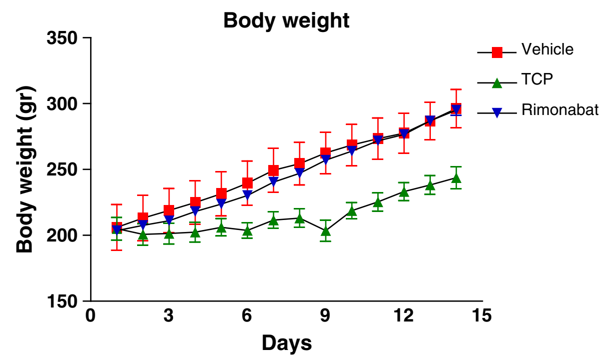


Fig. 1. Effect of chronic treatment with TCP (10 mg/kg/day i.p.) or rimonabant (2 mg/kg/day i.p.) compared to vehicle-treated rats on body weight over 14 days. Data are presented as means ± SEM of body weights during 14 days of treatment and analysed using two-way RM ANOVA. TCP (10 mg/kg/day) significantly reduced body weight (F (2, 294) = 3.74, p = 0.0408, n = 8) compared with vehicle-treated rats but rimonabant (10 mg/kg/day) had no effect on body weight over 14 days of treatment. All groups showed significant increases in body weight with time (F (14, 294) = 1050.37; $p<0.0001$, n = 8).

3.3. Effect of chronic treatment with TCP (10 mg/kg/day) or rimonabant (2 mg/kg/day) on BDNF and CREB/p-CREB protein expression in the frontal cortex, hippocampus, striatum and cerebellum

Chronic administration of TCP (10 mg/kg/day i.p.) significantly increased ($p<0.05$) BDNF protein expression in the frontal cortex compared with vehicle controls while rimonabant (2 mg/kg/day i.p.) had no effect (Fig. 6). There was no effect on the ratio of CREB: p-CREB in the frontal cortex following either drug treatment (Fig. 7). Neither TCP nor rimonabant-treated rats showed significant changes in BDNF expression levels or p-CREB ratios in the hippocampus, striatum and cerebellum.

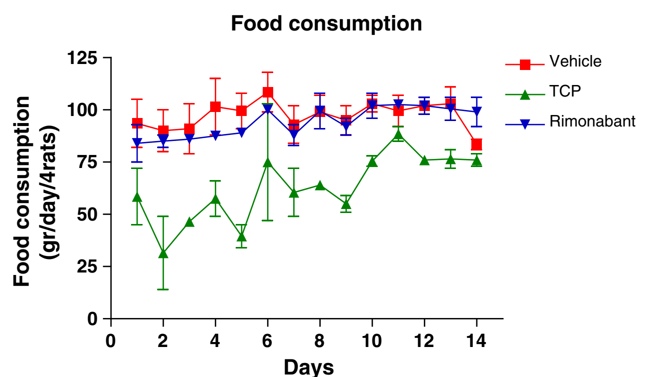


Fig. 2. Effect of chronic treatment with TCP (10 mg/kg/day i.p.) or rimonabant (2 mg/kg/day i.p.) compared to vehicle treated rats on food consumption during 14 days of experiment. Data are presented as mean ± SEM of body weight during 14 days of treatment and analysed using two-way RM ANOVA. All groups showed significant increases in food consumption with time (F (13, 39) = 4.76, p = <0.0001, n = 8) also TCP (10 mg/kg/day) significantly reduced food consumption (F (2, 39) = 13.55, p = 0.0315, n = 8) compared with vehicle rats but rimonabant (10 mg/kg/day) had no effect on food consumption.

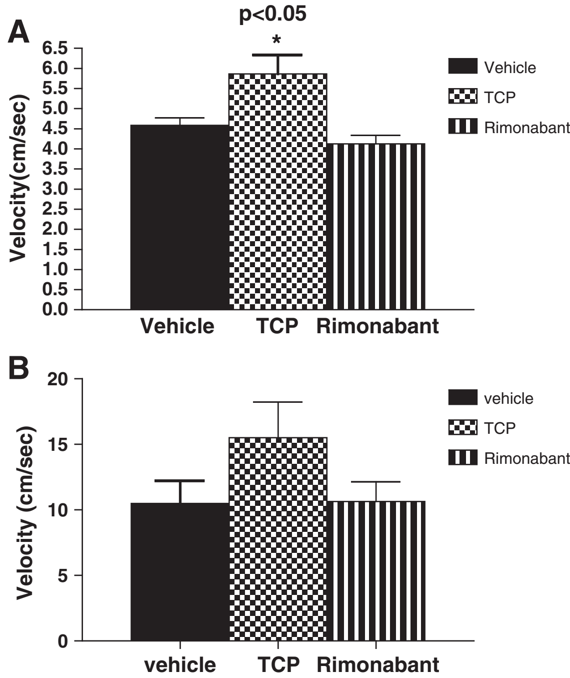


Fig. 3. Effect of treatment with TCP (10 mg/kg/day) or rimonabant (2 mg/kg/day) compared to vehicle treated rat on velocity on the 3rd (A) and 9th (B) days of treatment. Data are presented as mean ± SEM of velocity during 20 min. Data were analysed using One Way ANOVA and followed by Dunnett's Multiple Comparison Test in case of significance (n = 8 in all groups). TCP significantly increased velocity on the 3rd day of treatment compared with vehicle treated control but rimonabant had no effect. Neither TCP nor rimonabant affected the velocity on the 9th day of treatment compared with vehicle treated control.

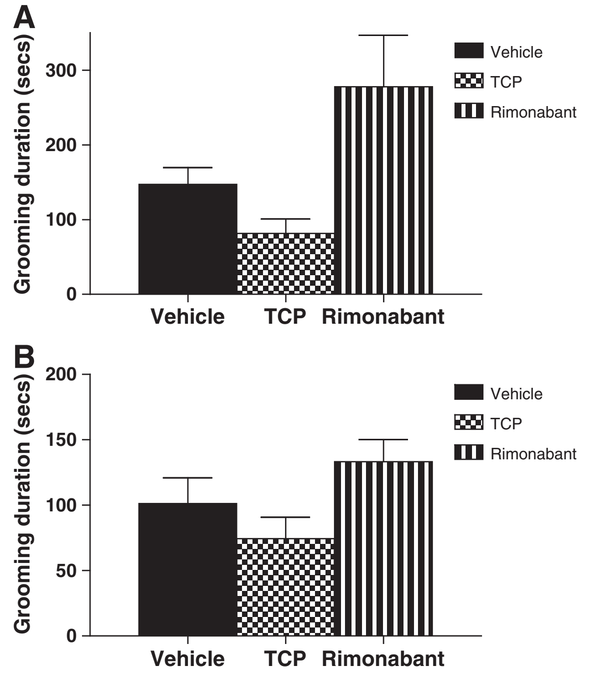


Fig. 5. Effect of treatment with of TCP (10 mg/kg/day) or rimonabant (2 mg/kg/day) compared to vehicle treated rat on grooming duration in the 3rd (A) and 9th (B) days of treatment. Data are presented as mean ± SEM of grooming duration over 20 min. Data were analysed using One Way ANOVA and followed by Dunnett's Multiple Comparison Test in case of significance (n = 8 in all groups). Neither TCP nor rimonabant significantly affected on grooming duration on the 3rd and the 9th days of treatment compared with vehicle treated control.

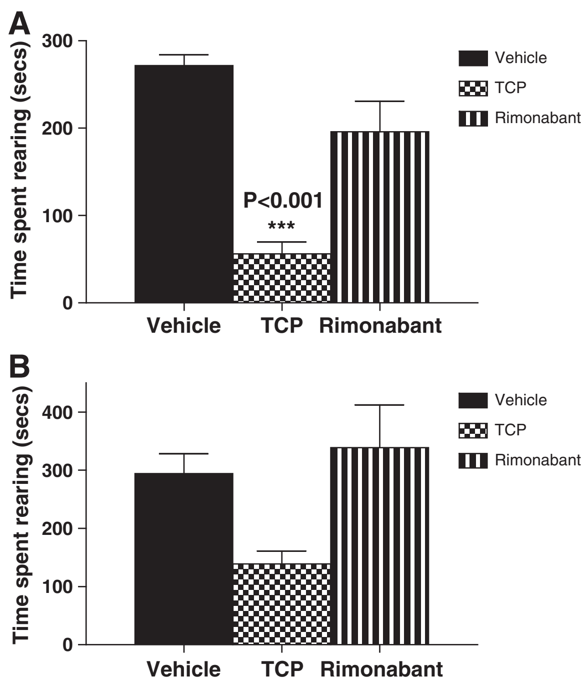


Fig. 4. Effect of treatment with TCP (10 mg/kg/day) or rimonabant (2 mg/kg/day) compared to vehicle treated rat on time spent rearing on the 3rd (A) and 9th (B) days of treatment. Data are presented as mean ± SEM of time spent rearing during 20 min. The data were analysed using One Way ANOVA and followed by Dunnett's Multiple Comparison Test in case of significance (n = 8 in all groups). TCP significantly decreased ($p < 0.001$) time spent rearing on the 3rd day of treatment compared with vehicle treated control but no change was observed in rimonabant treated rats. Neither TCP nor rimonabant affected the time spent rearing on the 9th day.

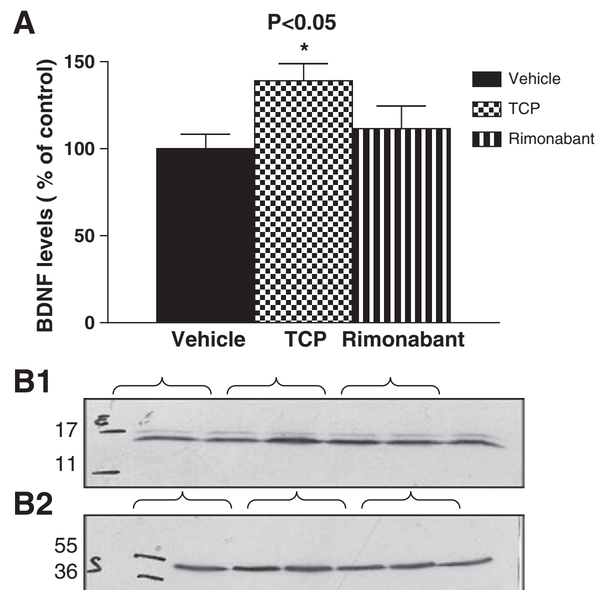


Fig. 6. Effect of chronic (14 days) treatment on BDNF protein levels in the cytosolic fraction of frontal cortex homogenate samples measured by western blot analysis of TCP (10 mg/kg/day) or rimonabant (2 mg/kg/day) vehicle treated rats. Data are presented as percent of control (100%) and are mean ± SEM with One Way ANOVA (n = 8 in all groups). A: Densitometric analysis, there was a significant increase in BDNF expression in the frontal cortex of TCP treated rats compared with vehicle control rats but rimonabant had no effect. B1: Shows BDNF band which is expected ~14 kDa weight. B2: Shows β-actin band with ~43 kDa weight indicating equal loading of our samples in wells.

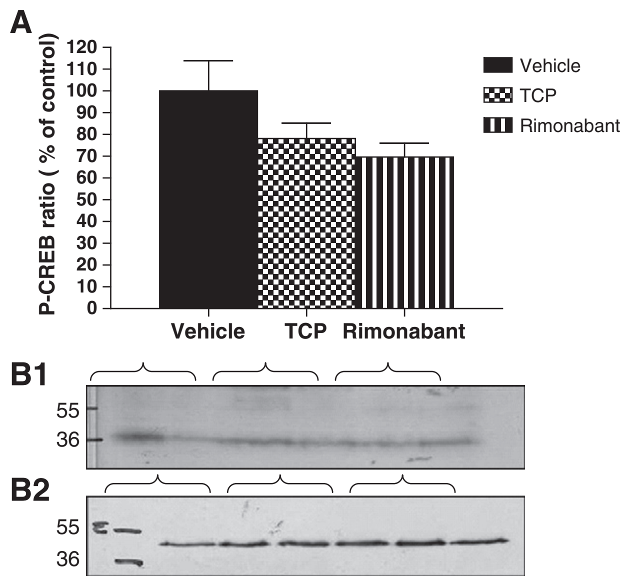


Fig. 7. Effect of chronic (14 days) treatment on p-CREB ratio protein in the nuclear fraction of frontal cortex homogenate samples measured by western blot analysis of TCP (10 mg/kg/day) or rimonabant (2 mg/kg/day) and vehicle treated rats. Data are presented as percent of control (100%) and are mean ± SEM with One Way ANOVA (n = 8 in all groups). A: Densitometric analysis, there was no significant changes in p-CREB ratio expression in the frontal cortex of drug treated groups compared with vehicle treatment (vehicle: 100.0 ± 13.81, n = 8, TCP: 78.19 ± 7.017, n = 8, rimonabant: 69.64 ± 6.420, n = 8). B1: Shows p-CREB band which is expected ~43 kDa weight. B2: Shows total CREB band with ~43 kDa weight.

4. Discussion

The present study showed that a conventional antidepressant TCP significantly reduced both body weight and food consumption; effects not observed with rimonabant. TCP also significantly increased locomotor velocity and reduced rearing on the 3rd day of the experiment. These changes in behaviour were accompanied by increased BDNF levels in the hippocampus of TCP-treated rats, as expected after chronic antidepressant drug treatment (Nibuya et al., 1995, 1996) while rimonabant failed to alter either behaviour or BDNF levels.

Food consumption and body weight were significantly reduced by TCP (10 mg/kg i.p.) over the 14 day experiment while rimonabant (2 mg/kg) had no effect on either parameter. In humans one of the most common effects of marijuana is stimulation of appetite (Abel, 1975) and it has been reported that use of cigarettes containing Δ^9-THC increases palatable food consumption (Foltin et al., 1988). It has previously been reported that TCP reduced the body weight of rats (Dulloo and Miller, 1984) and activation of CB1 receptors by endocannabinoids is considered to be important in the central hypothalamic control of appetite (Di Marzo and Matias, 2005). Cannabinoid receptor agonists may induce feeding by increasing the motivational or rewarding properties of food through an interaction with the endogenous opioid system (Manzanares et al., 1999). Furthermore, it has been postulated that the hyperphagia induced by anandamide is reduced by rimonabant indicating a role for endogenous cannabinoids in food consumption. It is worth noting that BDNF has a role in the regulation of body weight; for example, a significant reduction of BDNF was observed in the fasting human (Xu et al., 2003). Administration of BDNF to wild-type mice induced a loss of body weight via reduced food consumption (Pelleymounter et al., 1995). This interaction might be influenced by BDNF effecting either hypothalamic development or regulation of neurotransmitters involved in hypothalamic feeding circuits (Kernie et al., 2000). As BDNF has a vital role in long term potentiation in learning and memory, the other possibility is that BDNF produces a similar effect on hypothalamic feeding circuits in the regulation of feeding behaviour (Gray et al., 2006). In the present study, however, rimonabant did not significantly reduce body weight, food consumption or BDNF while TCP-treated rats showed a significant reduction in body weight and food intake while BDNF was increased which may indicate a role for BDNF in feeding behaviour. An explanation for the lack of effect of the CB1 antagonist may relate to the duration of treatment as a previous study found that subchronic daily treatment with rimonabant (1 mg/kg, 10 days) reduced food consumption and body weight gain in Zucker obese rats but importantly not in lean Zucker rats (Serrano et al., 2008). Furthermore another study found that food consumption and body weight of mature rats was reduced in a dose-dependent manner by daily injections of rimonabant (2.5 or 10 mg/kg) but this anorectic effect ceased after 5 days of treatment, possibly due to the development of tolerance, while the reduction in body weight was maintained throughout the experiment (Colombo et al., 1998). Such tolerance may account for the lack of effect on food intake over the 14 day treatment period using normal weight Lister hooded rats used in the present study.

Based on the literature we expected that rimonabant would affect locomotor activity, however it had no effect on the either the 3rd or 9th day of treatment and only showed a non-significant tendency to induce some anxiogenic-like effects, such as increased grooming (Fig. 5.A). A previous study has shown that rimonabant can induce anxiety in Wistar rats (Kathuria et al., 2003). It is however well established that there are rat strain differences in response to anxiogenic stimuli over a range of different behavioural tasks used to measure anxiogenic responses (Ennaceur et al., 2005) and it is possible that the Lister hooded rat is less susceptible to the reported aversive properties of rimonabant than Wistar rats. Moreover some studies have observed anxiolytic-like effect of rimonabant in some models of anxiety (Haller et al., 2002; Rodgers et al., 2003; Griebel et al., 2005).

The results revealed no significant changes in total distance moved following either TCP or rimonabant. A previous study with acute injections of rimonabant (0.1, 0.3, 1, 3 and 10 mg/kg) showed an increase in the awake-state EEG but no motor effects (Santucci et al., 1996). In contrast, TCP on the 3rd day increased movement velocity and there was a clear tendency for a similar increase on the 9th but it was not a significant effect. Rimonabant had no effect on locomotor activity on either the 3rd or 9th day. In CB1 knockout mice, locomotor activity is reduced compared to wild type which indicates a role for the endocannabinoid system in movement control (Zimmer et al., 1999). TCP-treatment reduced rearing after 3 days of treatment but had no effect after 9 days. Rearing and grooming are considered to be indices of anxiety-related behaviours mediated by the monoamines serotonin and noradrenaline (Moody et al., 1988) in the amygdala (Amaral, 2002; Bremner et al., 1996; Iversen, 1984). The decrease in rearing observed after 3 days of treatment with TCP may be indicative of an increase in aversive behaviour after acute treatment. Increased activity of the central noradrenaline and serotonin systems is considered to be essential for coping with aversive stimuli associated with fear and anxiety-related behaviours (Fanselow, 1980; Stanford and Marsden, 2005). Chronic TCP treatment has been shown to enhance release of both noradrenaline and serotonin in the hippocampus and frontal cortex of treated rats (Ferrer and Artigas, 1994; ZINI et al., 2000). In the present study TCP reduced rearing after 3 days of treatment while tending to decrease freezing behaviour induced by CER after 9 days of TCP treatment indicating an anxiolytic effect. Rimonabant had no effect on rearing or freezing after either acute or chronic treatment. The duration of freezing in the conditioned emotional response test is also used as an index of induced fear and anxiety (Maki et al., 2000). It has been reported previously that acute treatment with TCP (3–15 mg/kg) reduced freezing behaviour in rats (Duman, 1998; Lesch, 2001).

Chronic treatment with antidepressant drugs increases monoamine neurotransmitter functions which can then activate cAMP

and phosphoinositide hydrolysis pathways and, consequently, CREB phosphorylation resulting in the enhancement of the expression of neuroprotective genes such as BDNF (Ghosh and Greenberg, 1995; Nibuya et al., 1996). For example; the activation of second messenger signal transduction may occur by enhancing 5-HT and NE neurotransmission and activation of their receptors which are coupled to the cAMP-PKA cascade (*e.g.* 5-$HT_{4,6,7}$ and β-adrenoceptors) or by binding to other receptors (*e.g.* 5-HT_2 and α1-adrenoceptors) with subsequent activation of Ca^{2+}-dependent protein kinase (Ghosh and Greenberg, 1995; Nibuya et al., 1996). In the present study, neither TCP nor rimonabant had a significant effect on CREB expression although chronic TCP but not rimonabant increased BDNF levels in the frontal cortex as predicted for an antidepressant drug (Nibuya et al., 1996). However, Nibuya found an increased level of CREB expression in the hippocampus of antidepressant-treated rats while Coppell et al. (2003) suggested that the effect of antidepressant drugs such as TCP on BDNF gene expression in the hippocampus of rats is bi-phasic and time-dependent. So, the lack of effect of TCP on p-CRB/CREB expression in the present study might reflect such a dual action of the antidepressant in brain regions.

It is worth noting that, in previous studies, BDNF expression was only increased after chronic but not acute antidepressant drug administration (Pi-Sunyer et al., 2006) indicating an essential role of time as an important element in the therapeutic effect of antidepressant drugs.

It has been reported that Δ^9-THC and endocannabinoids inhibit neurotransmitter release partly by reducing activity in the cAMP pathway. However the present results showed no effects of rimonabant on BDNF or CREB/P-CREB expression and also no effect in the behavioural tests. Taken together these results do not support an antidepressant-like profile for this drug. As discussed previously, this is possibly no surprise given the reported incidence of depressive symptoms in subjects treated with rimonabant in the RIO, rimonabant in obesity, trials (US Food and Drug Administration Advisory Committee, 2007) and begs the question of whether the reports of antidepressant-like activity with novel CB1 antagonists in preclinical experiments were misleading, possibly because they were based on acute and not chronic administration of the drugs. Indeed, elevation of endocannabinoid tone using fatty acid amide hydrolase inhibitors has been reported to produce antidepressant-like effects in rodents (Bortolato et al., 2007; Adamczyk et al., 2008). However, it is interesting to note that chronic administration of TCP has been shown to reduce the levels of the endocannabinoid anandamide while increasing CB1 receptor density in rat hippocampus and frontal cortex (Hill et al., 2008). These results indicate that changes in monoamine neurotransmitter activity induced by antidepressant drugs can alter cannabinoid function and that such effects may be relevant to the antidepressant effects of drugs such as TCP.

In summary, it has been proposed that rimonabant might induce antidepressant-like effects. However, the present results showed no significant changes in normal rats consistent with such effects following rimonabant treatment. Witkin et al. (2005) proposed that CB1 receptor antagonists might exert antidepressant-like effects by altering the CB1 receptor regulation of the HPA axis. Therefore, as suggested by Witkin (2005), the therapeutic effects of rimonabant and other CB1 receptor antagonists might be dependent on the level of endocannabinoid tone. This indicates that modulation of the stress axis is more likely to be effective under conditions of stress rather than normal conditions and future studies should focus on the effects of CB1 agonists and antagonists using appropriate animal models of stress and depression.

Acknowledgements

The authors thank Liauqh Latif, Clare Spicer and Ian Topham for their kind technical help with the study and also the University of Nottingham and Egyptian Cultural Bureau for their financial support.

References

Abel EL. Cannabis: effects on hunger and thirst. Behav Biol 1975;15:255–81.

Adamczyk P, Golda A, Mccreary AC, Filip M, Przegalinski E. Activation of endocannabinoid transmission induces antidepressant-like effects in rats. J Physiol Pharmacol 2008;59:217–28.

Altar CA. Neurotrophins and depression. Trends Pharmacol Sci 1999;20:59–62.

Amaral DG. The primate amygdala and the neurobiology of social behavior: implications for understanding social anxiety. Biol Psychiatry 2002;51:11–7.

Animal (Scientific Procedures) Act 1986. London: Her Majesty's Stationery Office, 1986.

Baker D, Pryce G, Giovannoni G, Thompson AJ. The therapeutic potential of cannabis. Lancet Neurol 2003;2:291–8.

Bortolato M, Mangieri RA, Fu J, Kim JH, Arguello O, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biol Psychiatry 2007;62:1103–10.

Bremner JD, Krystal JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety: I. Preclinical studies. Synapse 1996;23:28–38.

Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet 2007;370:1706–13.

Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR 141716. Life Sci 1998;63:PL113–7.

Coppell AL, Pei Q, Zetterström TSC. Bi-phasic change in BDNF gene expression following antidepressant drug treatment. Neuropharmacology 2003;44:903–10.

Di Marzo V, Matias I. Endocannabinoid control of food intake and energy balance. Nat Neurosci 2005;8:585–9.

Dulloo AG, Miller DS. Thermogenic drugs for the treatment of obesity: sympathetic stimulants in animal models. Br J Nutr 1984;52:179.

Duman RS. Novel therapeutic approaches beyond the serotonin receptor. Biol Psychiatry 1998;44:324–35.

Ennaceur A, Michalikova S, Bradford A, Ahmed S. Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. Behav Brain Res 2005;159:247–66.

Fanselow MS. Conditional and unconditional components of post-shock freezing. Integr Psychol Behav Sci 1980;15:177–82.

Ferrer A, Artigas F. Effects of single and chronic treatment with tranylcypromine on extracellular serotonin in rat brain. Eur J Pharmacol 1994;263:227–34.

Foltin RW, Fischman MW, Byrne MF. Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. Appetite 1988;11:1-14.

French ED. [Delta] 9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. Neurosci Lett 1997;226:159–62.

Ghosh A, Greenberg ME. Calcium signaling in neurons: molecular mechanisms and cellular consequences. Science 1995;268:239–47.

Gonzalez S, Fernandez-Ruiz J, Marzo VD, Hernandez M, Arevalo C, Nicanor C, Cascio MG, Ambrosio E, Ramos JA. Behavioral and molecular changes elicited by acute administration of SR141716 to [Delta]9-tetrahydrocannabinol-tolerant rats: an experimental model of cannabinoid abstinence. Drug Alcohol Depend 2004;74:159–70.

Gray J, Yeo GSH, Cox JJ, Morton J, Adlam ALR, Keogh JM, Yanovski JA, El Gharbawy A, Han JC, Tung Y. Hyperphagia, severe obesity, impaired cognitive function, and hyperactivity associated with functional loss of one copy of the brain-derived neurotrophic factor (BDNF) gene. Diabetes 2006;55:3366.

Griebel G, Stemmelin J, Scatton B. Effects of the cannabinoid CB1 receptor antagonist rimonabant in models of emotional reactivity in rodents. Biol Psychiatry 2005;57:261–7.

Haller J, Bakos N, Szirmay M, Ledent C, Freund TF. The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. Eur J Neurosci 2002;16:1395–8.

Hill MN, Gorzalka BB. Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? Behav Pharmacol 2005;16:333–52.

Hill MN, Ho WSV, Hillard CJ, Gorzalka BB. Differential effects of the antidepressants tranylcypromine and fluoxetine on limbic cannabinoid receptor binding and endocannabinoid contents. J Neural Transm 2008;115:1673–9.

Iversen SD. 5-HT and anxiety. Neuropharmacology 1984;23:1553–60.

Kathuria S, Gaetani S, Fegley D, Vali o F, Duranti A, Tontini A, Mor M, Tarzia G, Rana GL, Calignano A. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med 2003;9:76–81.

Kelsey JE, Calabro S. Rimonabant blocks the expression but not the development of locomotor sensitization to nicotine in rats. Psychopharmacology 2008;198:461–6.

Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. EMBO J 2000;19:1290–300.

Lesch KP. Serotonergic gene expression and depression: implications for developing novel antidepressants. J Affect Disord 2001;62:57–76.

Lowry O, Rosebrough NJ, Rarr AL, Randal RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.

Maki Y, Inoue T, Izumi T, Muraki I, Ito K, Kitaichi Y, Li X, Koyama T. Monoamine oxidase inhibitors reduce conditioned fear stress-induced freezing behavior in rats. Eur J Pharmacol 2000;406:411–8.

Manji HK, Drevets WC, Charney DS. The cellular neurobiology of depression. Nat Med 2001;7:541–7.

Manzanares J, Corchero J, Romero J, Fernández-Ruiz JJ, Ramos JA, Fuentes JA. Pharmacological and biochemical interactions between opioids and cannabinoids. Trends Pharmacol Sci 1999;20:287–94.

Mendiguren A, Pineda J. Systemic effect of cannabinoids on the spontaneous firing rate of locus coeruleus neurons in rats. Eur J Pharmacol 2006;534:83–8.
Moody TW, Merali Z, Crawley JN. The effects of anxiolytics and other agents on rat grooming behaviora. Ann N Y Acad Sci 1988;525:281–90.
Murphy LL, Munoz RM, Adrian BA, Villanua MA. Function of cannabinoid receptors in the neuroendocrine regulation of hormone secretion. Neurobiol Dis 1998;5: 432–46.
Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 1995;15:7539–47.
Nibuya M, Nestler EJ, Duman RS. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 1996;16:2365–72.
Patel S, Hillard CJ. Role of endocannabinoid signalling in anxiety and depression. In: Kendall DA, Alexander S, editors. Current topics in behavioral neuroscience: behavioral neurobiology of the endocannabinoid system. Berlin: Springer-Verlag; 2009. p. 347–71.
Pelleymounter MA, Cullen MJ, Wellman CL. Characteristics of BDNF-induced weight loss. Exp Neurol 1995;131:229–38.
Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. JAMA 2006;295:761.
Rodgers RJ, Haller J, Halasz J, Mikics E. 'One trial sensitization'to the anxiolytic like effects of cannabinoid receptor antagonist SR141716A in the mouse elevated plus maze. Eur J Neurosci 2003;17:1279–86.
Rodriguez De Fonseca FR, Villanua MA, Munoz RM, San-Martin-Clark O, Navarro M. Differential effects of chronic treatment with either dopamine D1 or D2 receptor agonists on the acute neuroendocrine actions of the highly potent synthetic cannabinoid HU-210 in male rats. Neuroendocrinology 1995;61:714–21.
Rodriguez De Fonseca F, Rubio P, Menzaghi F, Merlo-Pich E, Rivier J, Koob GF, Navarro M. Corticotropin-releasing factor (CRF) antagonist [D-Phe12,Nle21,38,C alpha MeLeu37]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. J Pharmacol Exp Ther 1996;276:56–64.
Santucci V, Storme J-J, Soubrie P, Le Fur G. Arousal-enhancing properties of the CB1 cannabinoid receptor antagonist SR 141716A in rats as assessed by electroencephalographic spectral and sleep–waking cycle analysis. Life Sci 1996;58:PL103–10.
Serrano A, Del Arco I, Javier Pavón F, Macías M, Perez-Valero V, Rodríguez De Fonseca F. The cannabinoid CB1 receptor antagonist SR141716A (Rimonabant) enhances the metabolic benefits of long-term treatment with oleoylethanolamide in Zucker rats. Neuropharmacology 2008;54:226–34.
Skolnick P, Legutko B, Li X, Bymaster FP. Current perspectives on the development of non-biogenic amine-based antidepressants. Pharmacol Res 2001;43:411–23.
Stanford SC, Marsden CA. 5 Regional specialisation in the central noradrenergic response to unconditioned and conditioned environmental stimuli. Tech. Behav Neural Sci 2005;15:487–501.
US Food and Drug Administration Advisory Committee. FDA briefing document: Zimulti (rimonabant) tablets, 20 mg. available at: Rockville: FDA; 2007 http://www.fda.gov/OHRMS/DOCKETS/AC/07/briefing/2007-4306b1-00-index.htm (assessed 9 April, 2010).
Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. Science 2002;296: 678–82.
Witkin JM, Tzavara ET, Davis RJ, Li X, Nomikos GG. A therapeutic role for cannabinoid CB1 receptor antagonists in major depressive disorders. Trends Pharmacol Sci 2005;26:609–17.
Xu B, Goulding EH, Zang K, Cepoi D, Cone RD, Jones KR, Tecott LH, Reichardt LF. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 2003;6:736.
Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI. Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. Proc Natl Acad Sci 1999;96:5780–5.
Zini R, Tillement J, Morin D. Effect of antidepressants on extracellular catecholamine concentrations in the rat hippocampus using in vivo microdialysis. Biol Santé 2000;1:6-13.