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Repeated cannabinoid administration increases indices of noradrenergic activity in rats

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Abstract

The present study examined the impact of repeated administration of a synthetic cannabinoid agonist, WIN 55,212-2 on the coeruleo-cortical pathway, a circuit implicated in anxiety. Male Sprague–Dawley rats received repeated systemic injections of WIN 55,212-2 (3.0 mg/kg). A separate group of rats received repeated WIN 55,212-2 injections followed by a period of abstinence. Control animals received vehicle injections. Ninety minutes following the last injection on day 8, anxiety-related behavior was assessed using the elevated plus maze. The abstinent group was tested after another 8 days. Following behavioral testing, brain tissue was extracted from the locus coeruleus (LC) and probed for tyrosine hydroxylase (TH) expression. In a separate group of animals, in vivo microdialysis was used to monitor extracellular norepinephrine efflux in the frontal cortex following repeated WIN 55,212-2 administration and following a period of abstinence.

Repeated administration of WIN 55,212-2 evoked an anxiogenic-like response that was accompanied by an increase in TH protein expression in the LC. A similar neurochemical profile was observed using in vivo microdialysis where an augmented increase in cortical norepinephrine efflux was identified in response to a systemic injection of WIN 55,212-2 on day 8. Anxiety-like behavior, catecholamine synthesizing enzyme levels and NE efflux returned to control values after 8 days of abstinence.

The present findings indicate that repeated administration of a synthetic cannabinoid receptor agonist induces transient anxiety-like behaviors that correlate with increases in catecholamine synthesizing enzyme expression in the LC and augmented norepinephrine efflux in response to a challenge injection of WIN 55,212-2.

Keywords: WIN 55,212-2; Elevated plus maze; Western blot; Tyrosine hydroxylase; Norepinephrine; CB1 receptor; Microdialysis

1. Introduction

Repeated administration of cannabinoid agonists has been associated with activation of the stress-response system, anxiety, depression, and cognitive impairment (Arendt and Munk-Jorgensen, 2004; Arnold et al., 2001; Berrendero and Maldonado, 2002; Degenhardt et al., 2001; Manzanares et al., 2004; Troisi et al., 1998). The neural substrates underlying these effects remain to be elucidated. The biogenic amine, norepinephrine (NE) has been implicated in many of the same central processes that are affected by cannabinoids. Previous data from our laboratory indicate that acute administration of the synthetic cannabinoid agonist, WIN 55,212-2 increases NE efflux in the frontal cortex and stimulates c-Fos expression in noradrenergic neurons of the locus coeruleus (LC) (Oropeza et al., 2005), suggesting an interaction of cannabinoids with the coeruleo-cortical pathway, a circuit involved in modulating higher cognitive function and mood. This system is implicated in setting the attentional mode, and is engaged as part of the stress response to facilitate arousal (Aston-Jones et al., 1991, 1999) and thus is a brain substrate likely to be involved in behavioral changes associated with cannabinoid use.

Previous studies have shown that cannabinoid receptor agonists and antagonists can induce both anxiolytic and anxiogenic responses depending on the dose administered and familiarity of the environment (Rodriguez de Fonseca et al., 1996). In one study, blockade of cannabinoid receptors using a CB1 antagonist, SR 141716A caused anxiety-like behavior in

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the elevated plus maze and in the defensive burying paradigm (Navarro et al., 1997). Other studies have shown that blocking anandamide hydrolysis (by inhibiting fatty acid amide hydrolase) results in anxiolytic effects in two different models of anxiety (Kathuria et al., 2003). These findings suggest a role for the endocannabinoids in modulation of behavior. The question remains, however, where do cannabinoids act to induce cellular adaptations that may result in behavioral changes such as anxiety? One potential neural substrate may be the coeruleo-cortical noradrenergic system. CB1 receptors are found on both LC cell bodies and on norepinephrine containing terminals in the FC (Oropeza et al., 2004, 2007). In the present study, we sought to determine whether repeated cannabinoid administration results in a potentiated noradrenergic response that may contribute to behavioral adaptations consistent with anxiety. We selected the dose of 3.0 mg/kg WIN 55,212-2 for the present studies based on a pilot experiment that suggested an anxiolytic effect at this dose (data not shown).

Brain noradrenergic systems regulate many of the behavioral functions that are affected in depression and anxiety disorders (Morilak and Frazer, 2004). Several lines of evidence point to a dysfunction in the noradrenergic system in these conditions (Leonard, 1997). Upregulation of the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of catecholamines, has been suggested to lead to changes in noradrenergic transmission that contribute to behavioral, cognitive, emotional and physiological manifestations of depression and anxiety (Persson et al., 1997; Sands et al., 2000; Van Bockstaele et al., 1999). Levels of TH are known to be upregulated in specific brain regions by chronic administration of drugs of abuse (Boundy et al., 1998; Shishido et al., 1997). However, the effects of chronic cannabinoid administration on TH expression have been relatively unexplored in adult rat or human. In the present study, anxiety-like behavior, catecholamine synthesizing enzyme expression and NE efflux were assessed in rats treated for 8 days with the synthetic cannabinoid agonist, WIN 55,212-2 and following a period of abstinence. Modulation of the coeruleo-cortical noradrenergic pathway may contribute to changes in attention, cognition and anxiety commonly observed following THC exposure as this circuit is involved in modulating these behaviors (Aston-Jones et al., 1984; Jouvet, 1969; Valentino et al., 1993). Activity of noradrenergic neurons can modulate the level of cortical arousal and attention to internal and external stimuli. Dysfunction in noradrenergic circuits has been implicated in the development of affective disorders such as anxiety and depression (Aston-Jones and Bloom, 1981; Aston-Jones et al., 1984; Foote et al., 1983). To clarify the role of cannabinoids in the development of anxiety and other dysphoric reactions as well as to provide some insight into the underlying mechanism of action of cannabinoids in the modulation of behavior, continued studies into the effects of cannabinoids on behavior are necessary.

2. Materials and methods

2.1. Animals

A total of sixty-three male Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) weighing 250–300 g were housed 2–3 per cage on a 12-hour light schedule in a temperature-controlled (20 °C) colony room. Rats were given free access to standard rat chow and water. All experimental procedures received approval from the Thomas Jefferson University Institutional Animal Care and Use Committee (IACUC) and all studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal numbers, pain and distress.

2.2. Drugs

The cannabinoid CB1 receptor agonist, WIN 55,212-2 (mesylate salt, Sigma-Aldrich, St Louis, MO) was prepared fresh daily by dissolving it in deionized water (1.5 mg/ml) containing 0.25% Tween and injected intraperitoneally at a volume of 2 ml/kg. Animals received a single daily injection for 8 days. To test whether changes in behavior, protein expression and NE efflux were transient, groups of animals were given WIN 55,212-2 (3.0 mg/kg) for 8 days, followed by an 8-day period of abstinence from drug treatment. All control animals received 0.25% Tween dissolved in saline. The chronic treatment/abstinent group began treatment 1 week prior to the chronic treatment group so that testing would occur on the same day for both groups.

2.3. Behavior

Behavior on the elevated plus maze (EPM, Coulbourn Instruments, Allentown, PA) was assessed 90 min or 8 days following the last injection. Extracellular NE remained elevated at this timepoint when WIN 55,212-2 was administered i.p. It is unlikely that rats would be experiencing acute withdrawal at 90-min post-injection given the relatively long half-life (24–36 h) of WIN 55,212-2 (Aceto et al., 2001). Lighting in the testing room was adjusted from normal level (~450 lx) to a dimmer setting of 150–210 lx, where open arm light levels were ~200 lx and closed arm light levels were ~160 lx. For behavioral measurement, each rat was placed on an open arm of the maze facing the center and their behavior was video recorded for 5 min. Following the test, the animal was either returned to its home cage for abstinence studies or euthanized for tissue extraction and Western blot analysis. The maze was cleaned with a dilute ethanol solution and dried between each subject. Anxiety-like behavior was determined by calculating the amount of time and number of entries each rat made into the open and closed arms and reported as a percentage of the total time or total number of entries. An arm entry was counted when the superior portion of the rat including the head and neck (cranial to the transverse plane or ventral midline), the shoulders (pectoral region), the forelimbs and forepaws, and the thoracic region (anterior to the rib cage) moved into an open or closed arm. This measure sufficiently improves the reliability of the test by including open arm risk assessment behaviors, namely stretch attend postures (i.e., the rat stretches forward without moving its hindlimbs and hindpaws) and head dips (i.e., leaning over the edges of the open arms) (Dawson and Tricklebank, 1995).

2.4. TH expression levels

Immediately following behavioral testing, rats were euthanized by decapitation following exposure to isoflurane. Brain tissue was rapidly isolated on ice from each animal. Using a trephine, the LC was excised. Each brain sample was homogenized using a pestle and extracted in RadioImmunoPrecipitation Assay (RIPA) lysis buffer (Current Protocols in Molecular Biology) containing protease inhibitors (Complete Mini, Roche Molecular Biochemicals, Indianapolis, IN) on ice for 20 min. Lysates were cleared by centrifugation at 13,000 rpm for 12 min at 4 °C. Supernatants were removed and diluted with an equal volume of 2X Sodium Dodecyl Sulfate (SDS) sample buffer (Invitrogen, Carlsbad, CA) containing dithiothreitol (DTT, Sigma, St. Louis, MO). Protein concentrations of the supernatants were quantified using the bicinchoninic acid (BCA) reagent (Pierce, Rockford, IL). Individual variability in protein expression levels within a treatment group was determined by loading equal amounts of protein (15 μg) from each rat within a group on a 4% to 12% Tris–Glycine gel and subsequently electrophoretically transferred to a membrane (Immobilon-P, Millipore, Bedford, MA). Blots were incubated in an antibody directed against TH (Immunostar, Hudson, WI) or β-actin and visualized using a Western blotting detection system (Western Breeze Chemiluminescent Kit, Invitrogen, Carlsbad, California). Following densitometry analysis using Unscan-IT software (Silk Scientific, Inc), one-way ANOVA was used to assess differences in protein expression levels across individual samples within a treatment group. For each treatment group, no significant difference was identified between the individual samples (data not shown). Therefore, 5 μg of protein was obtained from each sample within the same treatment group and pooled. Densitometry analysis was subsequently conducted on pooled samples and data are represented as fold change compared to control.

2.5. Microdialysis and high-pressure liquid chromatography

After an acclimation period of approximately 1 week, rats were administered either saline (control) or WIN 55,212-2 (3.0 mg/kg) daily for 7 days. On the 7th day, rats were anesthetized with isoflurane 2–3% (in air) and placed in a stereotaxic apparatus with the skull flat. A small burr hole was made in the skull centered at 3.2 mm anterior and ±0.7 mm lateral to bregma. The dura was removed and the microdialysis probe was slowly lowered 5.0 mm from the brain surface into the infralimbic and prelimbic areas of the frontal cortex (Plates 8–10, Paxinos and Watson (1986)) and secured with skull screws and dental acrylic. The inlet of the probe was connected to a fluid swivel (Instech Laboratories, Plymouth Meeting, PA) and the rat was placed into a cylindrical plexiglass container covered with bedding. Food and water were freely available. Artificial cerebrospinal fluid (aCSF: 174 mM NaCl, 1.7 mM $CaCl_2$, 0.9 mM Mg Cl_2, and 4 mM KCl) was continuously perfused through the probe at a rate of 1.5 μl/min by a microliter infusion pump (Harvard Pump '11' VPF Dual Syringe, Harvard Apparatus, Holliston, MA). Rats were allowed to recover overnight. On day 8, approximately 18 h following surgery, dialysate samples were collected every 20 min. Following 2 h of baseline sample collection, WIN 55,212-2 or v (control) was administered by intraperitoneal injection in a volume of 2 ml/kg. A second control group received vehicle injections for 7 days. On day 8, they received an injection of WIN 55,212-2. Dialysate samples were collected for 3-h post-injection and stored at −80 °C for subsequent analysis by HPLC-ED. At the conclusion of the experiment, rats were deeply anesthetized (60 mg/kg pentobarbital) and 2% pontamine sky blue dye (Alfa Aesar, Ward Hill, MA) was infused through the probe to mark its location. The rats were transcardially perfused with 10% formalin (FormaFresh, Fisher Scientific, Pittsburgh, PA), decapitated and the brains removed for subsequent histological verification of probe placement. The data were not included if the placement was outside the infralimbic and prelimbic areas of the frontal cortex. A second group of rats was used for abstinence studies and received dialysis probes on day 15. Rats received a challenge injection of WIN 55,212-2 as described above, on day 16.

For details regarding microdialysis probe construction and quantification of norepinephrine levels please see Page et al., 2005. Briefly, 15 μl dialysate samples were analyzed by high-pressure liquid chromatography with electrochemical detection (HPLC-ED).

2.6. Data analysis

Mean basal NE levels were compared between groups using one-way analysis of variance (ANOVA). The baseline value against which drug effect was compared was derived from the average of three samples just prior to injection. The overall effect of treatment on NE levels was assessed using two-way, repeated-measures ANOVA. The absolute amount of neurotransmitter measured in dialysates (pg/sample) was used as the dependent variable for assessment of within group effects. Basal values plus the next eight samples post-drug injection were used in this analysis. Because the timecourse of WIN 55,212-2 effects was different between groups, we also analyzed the area under the curve to further compare and describe the response. The summed effect of treatment over the course of an experiment was measured by determining the area under the curve (AUC) describing extracellular NE levels as a function of time. AUC values for each animal were determined from the 12 post-drug samples and averaged by group and were then compared to control (Ch Sal/Sal) using a one-way ANOVA followed by Dunnett's test for comparisons between control and experimental groups. All statistics were performed using JMP software (SAS Institute, Cary, NC).

3. Results

3.1. Effects of repeated cannabinoid administration and abstinence on behavior and TH expression

Repeated administration of the CB1 agonist, WIN 55,212-2 resulted in behavior that was consistent with anxiety i.e., a decrease in the percent time spent in the open arms and a

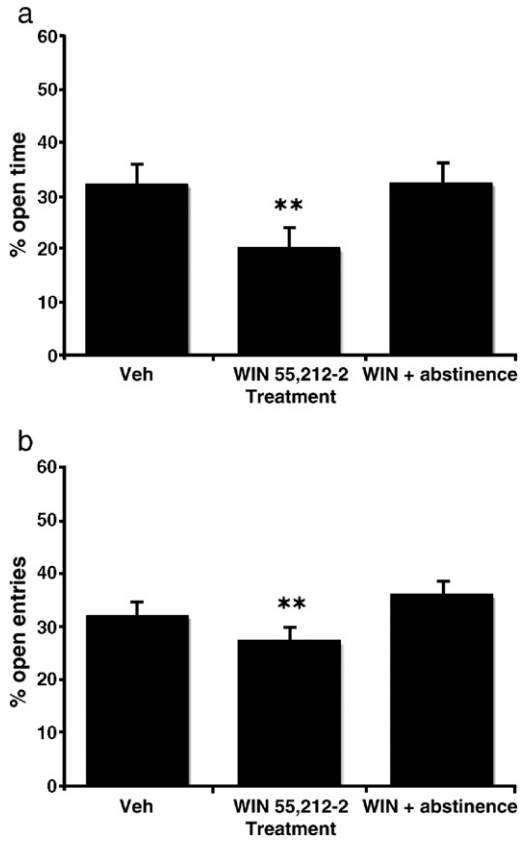

Fig. 1. Effects of WIN 55,212-2 on % open time (a) and % open entries (b) during a 5-min exposure to the elevated plus maze after repeated WIN 55,212-2 injections (3 mg/kg; $n=15$) and following an abstinence period ($n=14$). Control animals ($n=11$) received repeated injections of 0.25% Tween dissolved in saline. Animals were tested on the elevated plus maze for 5 min, 90 min after the final injection on day 8 or on the 8th day of abstinence. ANOVA revealed a significant effect of treatment for percentage open time; $^{**}p<0.01$, different from control and abstinent group and in the percentage open entries; $^{**}p<0.02$, different from abstinent group, Dunnett's post-hoc test.

decrease in the percent open entries (Fig. 1a,b). Samples of the LC probed for TH expression showed increases in catecholamine enzyme expression (Fig. 2) in rats that received repeated WIN 55,212-2 injections. To test whether changes in behavior and protein expression were transient, rats were given 3.0 mg/kg of WIN 55,212-2 for 8 days, followed by an eight-day period of abstinence from drug treatment. Figs. 1 and 2 compare the effect of repeated WIN 55,212-2 with repeated WIN 55,212-2 followed by abstinence. Control animals received repeated injections of vehicle. A significant difference was observed between groups for percentage time on open arms ($F[2,37]=4.78$; $p<0.01$; one-way ANOVA) and percentage entries on the open arms ($F[2,37]=4.18$; $p<0.02$; one-way ANOVA). There was a significant reduction in the total # of entries for the repeated WIN 55,212-2 group. Control animals made 13.3±1.1 total entries compared to 9.6± 0.93 for repeated WIN 55,212-2 treated animals and 11.5± 0.97 for abstinent animals ($F[2,37]=3.43$; $p<0.04$, one-way ANOVA). An over two fold increase in TH expression was observed in the LC following repeated systemic administration of WIN 55,212-2 (3.0 mg/kg) as compared to vehicle-treated

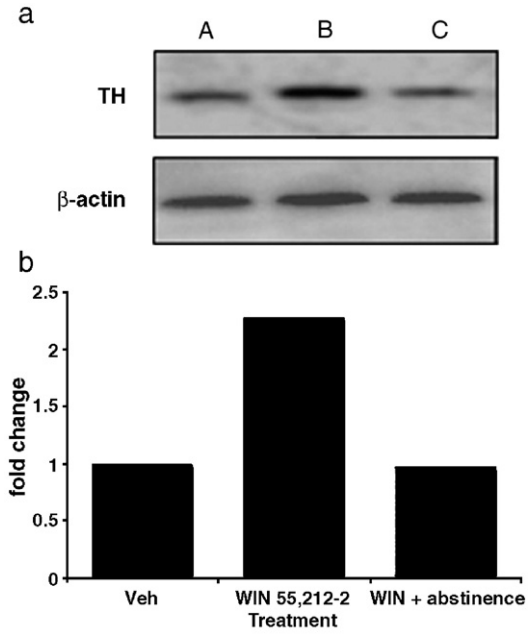

Fig. 2. Western blot analysis of TH protein expression in the LC. Animals repeatedly treated with WIN 55,212-2 (lane B) exhibited an over two fold increase in TH levels compared to vehicle-treated animals (lane A) and animals undergoing abstinence (lane C). TH expression levels of animals undergoing abstinence from WIN 55,212-2 treatment were comparable to vehicle-treated animals. Beta-actin immunoblotting was used as a control to verify equal protein loading. A=repeated saline; B=repeated WIN 55,212-2 treatment; C=repeated WIN 55,212-2+abstinence.

rats. Following a period of abstinence from drug administration, expression of TH was comparable to vehicle-treated rats (Fig. 2).

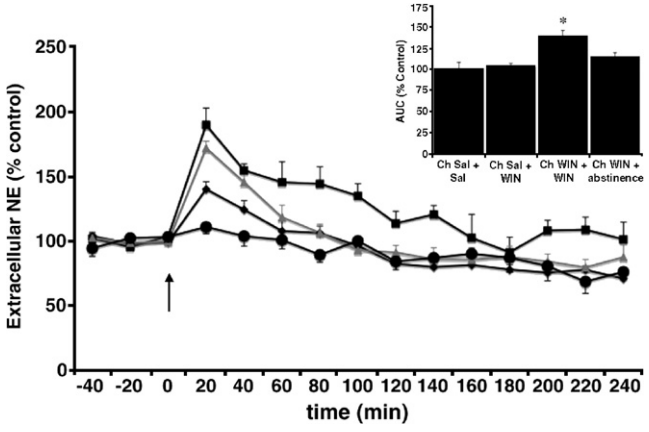

Fig. 3. Effects of repeated WIN 55,212-2 treatment on extracellular NE in the frontal cortex. Diamonds represent subjects that received repeated vehicle injection for 7 days and a challenge injection of WIN 55,212-2 on day 8 ($n=6$). Circles represent animals that received repeated vehicle injection for 7 days and a vehicle challenge injection on day 8 ($n=6$). Squares represent subjects that received repeated WIN 55,212-2 for 7 days and a challenge injection on day 8 ($n=6$); triangles represent subjects that received repeated WIN 55,212-2 injections for 7 days followed by 7 days of abstinence and a challenge injection on Day 15 ($n=7$). Values represent the mean±S.E.M. Samples were collected every 20 min. WIN 55,212-255,212-2 (or vehicle) was injected at the arrow. Inset illustrates the summed effect over time (AUC) for each group in response to WIN 55,212-2 injection compared to control (Ch Sal/Sal); One-way ANOVA, $^{*}p<0.05$.

3.2. Effect of repeated cannabinoid administration and abstinence on extracellular norepinephrine efflux

The effect of repeated administration of WIN 55,212-2 on extracellular NE in the frontal cortex is shown in Fig. 3. Two hours of baseline sample collection revealed that basal extracellular NE efflux was not different between rats that received repeated saline injections (1.56±0.03 pg/15 μl and 1.90±0.44 pg/15 μl), rats that received repeated WIN 55,212-2 injections (1.44±0.23 pg/15 μl) and rats that received repeated WIN 55,212-2 followed by abstinence (1.64±0.25 pg/15 μl) (F[3,20]=0.39, one-way ANOVA). Rats were challenged with a systemic injection of WIN 55,212-2 or saline after 2 h of baseline collection. The increase in NE in response to WIN 55,212-2 injection is illustrated in Fig. 3. Two way ANOVA showed no significant effect of treatment (F[3,20]=0.04; p=0.84) but a significant effect of time (F[8,160]=38.4; p<0.0001) and a significant time by treatment interaction (F[24,160]=3.03; p<0.0001). Analysis of the AUC revealed that the overall increase in extracellular NE in response to a single injection of WIN 55,212-2 was significantly greater in the rats that received repeated WIN 55,212-2 (F[3,19]=8.41; p<0.001, one-way ANOVA, see inset).

4. Discussion

Through the combination of behavioral, biochemical and neurochemical approaches, we describe the effects of repeated administration of the CB1 agonist, WIN 55,212-2 on indices of noradrenergic activity. Consistent with a wide body of literature describing both anxiolytic and anxiogenic effects of cannabinoids, our results indicate an anxiogenic effect of repeated administration of WIN 55,212-2 (3 mg/kg) that is accompanied by elevated TH levels in the LC. Correspondingly, a potentiated noradrenergic response to a challenge injection of WIN 55,212-2 was observed in rats that received repeated WIN 55,212-2 injections, suggesting a link between the noradrenergic system and anxiety-like behavior after repeated administration. Additionally, the alterations in TH expression and the response to challenge injection were found to be transient as these responses returned to baseline levels following 8 days of abstinence. We conducted initial studies examining the acute effects of WIN 55,212-2 on behavior in the EPM. In summary, we found an increase in the % time spent in the open arms with increasing doses of WIN 55,212-2 (0.3, 1.0 and 3.0 mg/kg). We also performed a chronic study with these 3 different doses. ANOVA showed a significant anxiolytic effect at 0.3 mg/kg and an opposite effect of 3.0 mg/kg, though this did not reach significance. We felt this was due to a small group size and chose to focus additional studies on only one dose (3.0 mg/kg).

4.1. Methodological considerations

Certain caveats exist with respect to Western Blot analysis experiments. These include the importance of accurate sampling of the region of interest and comparison of equal protein quantities across treatment groups. To minimize variability in tissue excision, a single investigator obtained the brain samples for each experiment. In addition, equivalent protein loading was verified using β-actin as a control and results were normalized to this internal standard. No significant difference in β-actin was detected across treatment groups.

4.2. Behavioral adaptation

Our results are consistent with others' findings showing that cannabinoid receptor agonists are capable of eliciting anxiogenic responses (Rodriguez de Fonseca et al., 1996). The effect of repeated WIN 55,212-2 administration on anxiety-like behaviors is consistent with several human studies in which a link between cannabinoid administration and anxiety has been reported (Gregg et al., 1976; Meyer et al., 1971; Zuardi et al., 1982). For example, acute administration of cannabinoids in humans can result in anxiety (Zuardi et al., 1982). However, others have reported that blockade of the CB1 receptor with SR 141716A induces an anxiety-like response in both the elevated plus maze and the defensive withdrawal test in rats (Navarro et al., 1997). Still, other studies have shown that inhibition of FAAH, the enzyme involved in the hydrolysis of anandamide, an endocannabinoid, causes anxiolytic responses in animal models of anxiety (Kathuria et al., 2003). To date, no biochemical correlate has been specifically linked to anxiety following repeated cannabinoid treatment. The present findings suggest that the coeruleo-cortical noradrenergic system might be one of the neural substrates involved in the modulation of anxiety by cannabinoid ligands. Aceto et al. (2001) showed mild, spontaneous withdrawal in rats treated with WIN 55,212-2 for 4 days with a relatively high dose regimen (2, 4, 8, 16 mg/kg) at 24-h post-infusion. Our treatment regimen was 3.0 mg/kg/day for 8 days and the behavioral assessment was conducted 90 min after the last injection. Thus, it is unlikely that the behavior would be related to acute withdrawal.

4.3. Effects on catecholamine synthesizing enzymes

One of the most consistent biochemical changes seen in response to chronic drug exposure is the upregulation of TH, the rate-limiting enzyme in the biosynthesis of the catecholamine neurotransmitters (Boundy et al., 1998). For example, chronic morphine administration has been shown to increase levels of TH and catalytic activity in the LC (Guitart et al., 1990). Upregulation of TH would be expected to increase the capacity of these neurons to synthesize norepinephrine, which could contribute to the dramatic increase in NE release seen in target regions of the LC and to associated behavioral changes during opiate withdrawal (Grasing et al., 1997; Koob et al., 1992).

TH is regulated in a complex modular fashion by both positive and negative regulatory elements (Liu et al., 1997). Previously published studies by others have shown that exposing pregnant rats to THC during the perinatal period affects the gene expression and the activity of TH in the brains of their offspring at peripubertal and adult ages (Bonnin et al., 1994, 1995, 1996; Hernandez et al., 2000). However, few studies have examined the impact of repeated cannabinoid administration on TH in adult

rats. Several lines of evidence suggest that cannabinoids may impact TH gene expression. Our previous study showed a significant increase in c-Fos expression in noradrenergic LC neurons following an acute systemic injection of a synthetic cannabinoid agonist. Expression of c-Fos is increased as a result of somatodendritic depolarization and is therefore a useful marker for detecting neuronal activation (Morgan and Curran, 1986). However, c-Fos activation has also been linked to regulation of catecholamine gene expression (Swanson et al., 1998). The evidence for this comes from studies indicating that cAMP activates effectors (e.g., PKA) that stimulate transcription through a shift in the AP1 binding components to include c-Fos. Induction of c-Fos binding as well as an increase in c-Jun/JunD (Jun) binding to the CRE-AP1 site may work to stabilize the interaction of the co-activator CBP and the transcription machinery with the promoter to enhance transcription of catecholamine synthesizing enzymes (Swanson et al., 1998).

4.4. Effects on norepinephrine efflux

The results from the microdialysis studies confirm that the noradrenergic neurons of the locus coeruleus are activated by systemic administration of synthetic cannabinoids at the dose of 3 mg/kg. Although our treatment regimen was of relatively short duration in terms of chronic drug use, we observed significant augmentation of the noradrenergic response to WIN 55,212-2 injection after 8 days of repeated administration. The increase in extracellular NE was greater and of a prolonged duration in this group, a profile consistent with an increase in anxiety (Page and Lucki, 2002; Page et al., 2005). Interestingly, the basal levels of NE did not differ among treatment groups. The response to WIN 55,212-2 injection was augmented in the group that received repeated injections. In the behavioral assay, abstinent rats were assessed on the elevated plus maze in the absence of any challenge injection and found to be similar to control rats. Because basal levels of NE did not differ between groups, we were interested to know whether the augmented response to a challenge injection of WIN 55,212-2 was maintained after a period of abstinence or if it returned to control values. Our findings demonstrated a return to the control level of response. This suggests that the alteration in noradrenergic function elicited by this repeated treatment schedule is subtle and is consistent with the transient nature of the changes observed in behavior and TH expression.

The present data are in agreement with studies showing that cellular and behavioral changes that occur following cannabinoid administration return to control levels after a period of abstinence (Gonzalez et al., 2004; Lichtman and Martin, 2002; Maldonado, 2002). This is an important issue as controversy exists regarding whether cannabinoids produce tolerance as is the case with many other abused substances (Kalant, 2004), such as morphine (Williams et al., 2001), alcohol (Weiss and Porrino, 2002) and cocaine (for review, see Dackis and O'Brien, 2001). Further studies examining the behavioral response to challenge doses of WIN 55,212-2 after repeated administration would be required before a conclusion could be made regarding the extent of behavioral adaptation.

Continued investigation into the effects of repeated administration of cannabinoid ligands on the coeruleo-cortical noradrenergic system is warranted. These studies should include longer duration of treatment and at varying doses and would likely further our understanding of the role of this system in mediating both positive and negative effects of drugs of abuse and may contribute to the development of future therapeutic applications.

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