Altered responsiveness of serotonin receptor subtypes following long-term cannabinoid treatment

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Abstract

This study examined the effects of long-term cannabinoid administration on the responsivity of 5-HT$_1$A and 5-HT$_2$A receptors, which have been implicated in depression. Animals received 12 d administration of the potent cannabinoid receptor agonist HU-210 (100μg/kg), following which they were monitored on their behavioural, physiological and hormonal responses to a single challenge of a 5-HT$_1$A and 5-HT$_2$A receptor agonist, 8-OH-DPAT (0.3 mg/kg) and DOI (1 mg/kg) respectively. Chronic HU-210 treatment lead to a significant enhancement of DOI-induced wet-dog shakes, but a reduction of DOI-induced back muscle contractions. DOI-induced corticosterone release was unaffected by HU-210 treatment. The hyperthermic response to DOI appeared to be potentiated by long-term HU-210 treatment, as 50% of these subjects died from an apparent serotonin syndrome with core temperatures exceeding 43°C. The 8-OH-DPAT-induced hypothermic response and elevation of corticosterone were both significantly attenuated by long-term HU-210 treatment. These data imply that chronic cannabinoid treatment may up-regulate 5-HT$_2$A receptor activity while concurrently down-regulating 5-HT$_1$A receptor activity, a finding similar to that sometimes observed in depression. This may partially explain the association between excessive cannabis consumption and the induction of affective disease.

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Introduction

The serotonin (5-HT) system is a diverse and intricate system composed of at least 14 identified receptor subtypes (Barnes and Sharp, 1999). Serotonergic nerve fibres originate in the raphe nuclei of the hindbrain and project diffusely throughout the brain, innervating almost every major brain structure (Abrams et al., 2004). One interesting aspect of the 5-HT system is the reciprocal interactions many of its receptors have with one another. For example, 5-HT$_1$A and 5-HT$_2$A receptors appear to exhibit opposing roles (Araneda and Andrade, 1991; Ashby et al., 1994; Darmani et al., 1990). Specifically, activation of 5-HT$_1$A receptors typically results in cellular hyperpolarization and inhibition of cell firing, whereas activation of 5-HT$_2$A receptors induces cellular depolarization and increased cell firing (Araneda and Andrade, 1991; Bobker, 1994; Craven et al., 2001). Concomitant activation of one of these receptors results in a functional inhibition of the other, suggesting that the net effect of serotonergic activity is delicately regulated by the balance of these two receptors. This is exemplified by the demonstration that the ability of 5-HT$_1$A receptor agonists to suppress the firing rate of spontaneously active cortical cells is potentiated by the concurrent administration of 5-HT$_2$A receptor antagonists, and the ability of 5-HT$_2$A receptor ligands to enhance glutamate-induced cell firing is counteracted by administration of 5-HT$_1$A receptor agonists (Ashby et al., 1994).

This reciprocal regulation of 5-HT$_1$A and 5-HT$_2$A receptors is also seen in behavioural research. Administration of ligands for the 5-HT$_1$A receptor attenuate the expression of 5-HT$_2$A receptor-mediated stereotypes such as wet-dog shakes, and administration of antagonists at the 5-HT$_1$A receptor potentiate 5-HT$_2$A receptor-mediated wet-dog shakes (Darmani et al., 1990, 1991; Willins and Meltzer, 1997). Furthermore, administration of 5-HT$_2$A receptor antagonists...
enhances the behavioural responses to administration of a 5-HT1A agonist (Backus et al., 1990). Additionally, these two receptors appear to elicit opposing behavioural responses, with 5-HT1A receptor activation inducing hyperphagia, increased male sexual behaviour, anxiolysis and hyperthermia whereas activation of the 5-HT2A receptor induces hyperthermia, reduced male sexual behaviour, anxiogenesis and hypophagia (Abdel-Fattah et al., 1995; Eison and Eison, 1994; Gorzalka et al., 1990; Simansky, 1996). This suggests a dynamic relationship exists between the 5-HT1A and 5-HT2A receptors that requires an optimal level of activity at both receptors, and that alterations in one receptor subtype will inevitably affect the other.

The endocannabinoid system is a neuromodulatory system in the brain, which shares a high level of overlap with the serotonergic system in terms of the physiological processes it regulates. For example, both the serotonergic and endocannabinoid systems regulate body temperature, feeding behaviour, sleep and arousal and emotional processes (Abdel-Fattah et al., 1995; Chaperon and Thiebot, 1999; Eison and Eison, 1994; Santucci et al., 1996; Simansky, 1996). Despite the extent of the similarity between these two systems, relatively little is known about their functional interactions. In-vitro and in-vivo work has suggested that cannabinoids may influence 5-HT release. Specifically, cannabinoid receptor (CB1) agonists suppress electrically and Ca2+-stimulated 5-HT release from cortical slices (Nakazi et al., 2000) and administration of CB1 receptor antagonists stimulates 5-HT release into the medial prefrontal cortex (Darmani et al., 2003; Tzavara et al., 2003). In the hippocampus, the psychoactive constituent of cannabis, delta9 tetrahydrocannabinol (THC) has been shown to inhibit 5-HT release (Egashira et al., 2002). This suppression of serotonergic neurotransmission by cannabinoids is believed to be involved in the mnemonic effects of THC as treatment with the 5-HT precursor, 5-hydroxytryptophan (5-HTP), or the 5-HT reuptake inhibitor clomipramine reversed this deficit (Egashira et al., 2002).

Thus, while cannabinoids may regulate serotonergic release and transmission, very little is known about the direct relationship between cannabinoids and specific 5-HT receptor subtypes. Biochemical work has suggested that endocannabinoids may enhance 5-HT1A receptor-mediated responses but attenuate 5-HT2A receptor-mediated responses (Boger et al., 1998), a finding partially supported by behavioural studies demonstrating that acutely, cannabinoid administration can reduce 5-HT1A receptor-mediated behavioural responses (Cheer et al., 1999; Darmani, 2001; Gorzalka et al., 2005). The effects of acute administration of cannabinoid ligands on 5-HT1A receptor-mediated behavioural responses remain to be determined.

To date there have been no studies examining the effect of chronic cannabinoid administration on the balance of 5-HT receptors. Using behavioural, physiological and hormonal measures of both 5-HT1A and 5-HT2A receptor responses, the present study examined the effect of chronic administration of the cannabinoid CB1 receptor agonist HU-210 on the responsiveness of 5-HT1A and 5-HT2A receptors.

**Methods**

**Subjects and treatment**

Seventy-day-old male Long–Evans rats (300 g) housed in groups of three in triple-mesh wire cages were used in this study. Colony rooms were maintained at 21 °C, and on a reverse 12 h light/dark cycle, with lights off at 09:00 hours. All rats were given ad libitum access to Purina rat chow and tap water. All subjects were randomly divided into two groups: (1) 100 μg/kg HU-210 (Tocris-Cookson, Bristol, UK); or (2) vehicle (1:1:18: Tween-80:dimethyl sulphoxide:0.9% saline). All injections were performed intraperitoneally using 26-gauge ½-inch needles, during the last third of the dark cycle. All subjects received either HU-210 or vehicle injections for 12 consecutive days. Twenty-four hours following the final injection, both groups were then divided into three subgroups: (1) vehicle (0.9% saline); (2) 0.3 mg/kg 8-hydroxy-2-(N,N-dipropylamino)tetratin (8-OH-DPAT; a 5-HT1A receptor agonist; Sigma-Aldrich, Oakville, ON, Canada); (3) 1 mg/kg ((1-2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; a 5-HT2A/2C receptor agonist; Sigma-Aldrich). These doses were based on previous psychopharmacological studies and are within normal testing range (Akiyoshi et al., 1995; Gorzalka et al., 1998; Hofmann et al., 2002; Mikklesen et al., 2004). Separate cohorts of animals were used for the hormonal studies (n=5 per group) and the behavioural/thermal (n=7 per group) studies. All experiments were performed under the ethical consent of the Animal Care Committee of the University of British Columbia and the Canadian Council on Animal Care.

**Behavioural and thermal testing procedures**

All behavioural and thermal testing began at the onset of the dark cycle and was performed by blind observers. Immediately prior to injection of saline, 8-OH-DPAT or DOI, and 30, 60 and 90 min post-injection, all subjects had their rectal temperature recorded.
measured with a Yellow Springs Instrument Series 400 thermister thermometer model 8402-00 (Yellow Springs, OH, USA). For each rectal temperature reading, rats were held at the base of the tail and the probe was inserted 5.2 cm past the rectum into the colon for 6–8 s until a rectal temperature was maintained for 3 s.

Animals administered saline or DOI were also monitored for behavioural stereotypies. Immediately following injection of DOI or saline, subjects were placed into a Plexiglas testing bin (60 cm × 45 cm × 45 cm) lined with Aspen chip bedding. During a 30-min interval in the testing bin, they were scored for the occurrence of wet-dog shakes (defined as a paroxysmal shudder of the head, neck and trunk; Bedard and Pycock, 1977; Gorzalka and Hanson, 1998) and back muscle contractions (defined as a clear-cut powerful contraction sweeping from the back of the neck along the back to the tail; Fone et al., 1989).

Plasma collection and radioimmunoassay
Following 12 d administration of HU-210 or vehicle, animals were again divided into three subgroups: (1) 0.9% saline; (2) 0.3 mg/kg 8-OH-DPAT; (3) 1 mg/kg DOI. Twenty-four hours following the last HU-210 or vehicle injection, subjects were administered an injection of saline, 8-OH-DPAT or DOI at the onset of the dark cycle (when the circadian level of corticosterone is at its trough), and returned to their home cages. Thirty minutes following injection, subjects were removed from their home cage and rapidly decapitated and their trunk blood was collected. Upon collection of trunk blood, samples were stored overnight at 4 °C and centrifuged the following morning at 10 000 rpm for 10 min. Serum was removed and centrifuged again at 10 000 rpm for 10 min and stored in a freezer at −20 °C until analysis. Serum was assayed for corticosterone levels with a standard radioimmunoassay kit and all samples were run in duplicate (Diagnostic Systems Laboratories Inc., Webster, TX, USA). Specifically, samples were thawed and aliquoted into polystyrene test tubes containing rat corticosterone standard. Rat [125]Icorticosterone reagent was immediately added followed by rat corticosterone antiserum, after which all samples were vortexed briefly and left to incubate in a water bath at 37 °C for 60 min. Following incubation, all samples had 1 ml of precipitating agent added and were vortexed briefly and left to incubate at room temperature (25 °C) for 15 min. All tubes were then centrifuged for 15 min at 3000 rpm. All tubes were then decanted in a sponge rack and blotted to remove all fluid. Tubes were then quantified in a gamma counter at 1 min per tube.

Statistical analysis
Data for behavioural and hormonal measures were analysed using a univariate analysis of variance (ANOVA) with serotonergic drug (8-OH-DPAT or DOI) and cannabinoid treatment as fixed factors. Data for thermal measurements were analysed using a repeated-measures ANOVA with both serotonergic drug and cannabinoid treatment as fixed factors. Tukey’s post-hoc test was performed on all data and significance levels were set at p < 0.05.

Results
Chronic cannabinoid treatment on 5-HT2A receptor-mediated responses
There was a significant interaction between DOI and HU-210 treatment on the expression of DOI-induced wet-dog shakes [F(1, 24) = 6.871, p < 0.02], with significant main effects of both DOI [F(1, 24) = 43.85, p < 0.001] and HU-210 treatment [F(1, 24) = 10.51, p < 0.01]. Post-hoc analysis demonstrated that DOI significantly increased wet-dog-shake expression compared to control treatment (p < 0.05), and that chronic pretreatment with HU-210 significantly potentiated DOI-induced wet-dog shakes (p < 0.01). Furthermore, chronic pretreatment with HU-210 alone did not alter basal (non-DOI) wet-dog-shake levels (p = 0.97). Data for the effect of chronic HU-210 pretreatment on DOI-induced wet-dog shakes can be seen in Figure 1a.

There was also a significant interaction between DOI and HU-210 treatment on the expression of DOI-induced back muscle contractions [F(1, 24) = 4.886, p < 0.05], with significant main effects of both DOI [F(1, 24) = 37.50, p < 0.001] and HU-210 treatment [F(1, 24) = 4.886, p < 0.05]. Post-hoc analysis demonstrated that DOI significantly increased back muscle contractions relative to control animals (p < 0.001), but chronic pretreatment with HU-210 significantly attenuated the magnitude of this effect (p < 0.05 relative to DOI alone, but p > 0.05 relative to the control group). Chronic HU-210 pretreatment alone did not affect basal levels of back muscle contractions (p = 1.0). Data for the effect of chronic HU-210 pretreatment on DOI-induced back muscle contractions can be seen in Figure 1b.

The effect of HU-210 pretreatment on DOI-induced hyperthermia could not be assessed in this experiment as animals that had been pretreated with HU-210 and were administered DOI exhibited pronounced signs of a 5-HT syndrome that was specifically characterized by a severe hyperthermia (core temperatures exceeded 43 °C in most subjects). By the 60-min time-point (third temperature measurement), over half of
these animals had unexpectedly died. Despite insufficient data for a repeated-measures analysis, these findings suggest that the hyperthermic response to DOI was potentiated by chronic HU-210 pretreatment. There was, however, no significant interaction between DOI and HU-210 treatment on DOI-induced increases in corticosterone secretion \( F(1, 16) = 0.082, \ p > 0.05 \); however, there was a significant main effect of DOI \( F(1, 16) = 99.65, \ p < 0.001 \), but no significant main effect of HU-210 treatment \( F(1, 16) = 0.052, \ p > 0.05 \). Data for the effect of chronic HU-210 pre-treatment on DOI-induced changes in corticosterone secretion can be seen in Figure 2.

Chronic cannabinoid treatment on 5-HT\(_{1A}\) receptor-mediated hypothermia

There was a significant interaction between time, 8-OH-DPAT and HU-210 treatment on the 8-OH-DPAT-mediated hypothermic response \( F(2, 48) = 3.17, \ p < 0.05 \). Furthermore, there was also a significant interaction between time and HU-210 treatment \( F(2, 48) = 4.45, \ p < 0.05 \), but not a significant interaction between time and 8-OH-DPAT treatment \( F(2, 48) = 1.02, \ p > 0.05 \). Post-hoc analysis demonstrated that 8-OH-DPAT treatment significantly reduced core temperature between time 0 and time 30 \( (p < 0.01) \). Animals that had been pretreated with HU-210 and received a challenge dose of 8-OH-DPAT did not differ in temperature changes from control animals \( (p = 0.41) \), and exhibited significantly less hypothermia than vehicle-pretreated animals also administered 8-OH-DPAT \( (p < 0.001) \). At 60-min post-injection, the hypothermic effect of the 8-OH-DPAT challenge in vehicle-pretreated animals continued to be significantly lower than vehicle-pretreated animals that received a vehicle challenge \( (p < 0.01) \). At the 60-min time-point, animals which had been pretreated with HU-210 and received a challenge dose of 8-OH-DPAT did not differ in temperature changes from control animals \( (p = 0.41) \), and exhibited significantly less hypothermia than vehicle-pretreated animals also administered 8-OH-DPAT \( (p < 0.001) \). At 60-min post-injection, the hypothermic effect of the 8-OH-DPAT challenge in vehicle-pretreated animals continued to be significantly lower than vehicle-pretreated animals that received a vehicle challenge \( (p < 0.01) \). At the 60-min time-point, animals which had been pretreated with chronic HU-210 and administered an 8-OH-DPAT challenge exhibited no significant change in core temperature relative to vehicle-vehicle-treated animals \( (p = 0.98) \) and exhibited significantly less hypothermia than vehicle-pretreated animals \( (p < 0.02) \). However, HU-210 pre-treatment followed by a vehicle challenge did result in a very small, but significant, elevation in core temperature at 60 min \( (p < 0.05) \). At 90 min, there was a non-significant trend towards a reduction in core temperature with 8-OH-DPAT administration in vehicle-pretreated animals \( (p < 0.09) \). Although HU-210 pre-treatment followed by a vehicle challenge had no effect on core temperature at this time-point \( (p > 0.05) \), HU-210 pretreated animals subsequently...
given a 8-OH-DPAT challenge exhibited significantly less hypothermia than vehicle-pretreated animals given a 8-OH-DPAT challenge (p < 0.01). Data indicating the time-course of the effect of chronic HU-210 pretreatment on the hypothermic effects of 8-OH-DPAT can be seen in Figure 3.

There was a significant interaction between 8-OH-DPAT and HU-210 treatment on the 8-OH-DPAT-induced increase in corticosterone secretion [F(1, 16) = 30.29, p < 0.001], with significant main effects of both 8-OH-DPAT treatment [F(1, 16) = 31.34, p < 0.001] and HU-210 treatment [F(1, 16) = 50.25, p < 0.001]. Post-hoc analysis revealed that 8-OH-DPAT administration significantly increased serum corticosterone levels relative to control levels (p < 0.001). However, following pretreatment with HU-210, 8-OH-DPAT administration failed to increase serum corticosterone levels relative to control levels (p = 0.72). Furthermore, 8-OH-DPAT administration to HU-210 pretreated animals resulted in a significantly smaller change in serum corticosterone than 8-OH-DPAT administration to vehicle-treated animals (p < 0.001). Chronic HU-210 treatment alone had no effect on serum corticosterone levels (p = 0.68). Data indicating the effect of chronic HU-210 pretreatment on the adrenocortical response to 8-OH-DPAT administration can be seen in Figure 4.

**Discussion**

This research demonstrated that prolonged treatment with the potent cannabinoid CB receptor agonist HU-210 (100 μg/kg, d) resulted in an apparent dysregulation of 5-HT receptor subtypes. Behaviourally, chronic cannabinoid treatment potentiated 5-HT_{2A} receptor-mediated wet-dog shakes, but decreased back muscle contractions. Furthermore, in animals that had been pretreated with chronic HU-210, a standard 1 mg/kg dose of DOI resulted in a sudden and unanticipated mortality in four of the seven subjects within 60 min from an apparent serotonin syndrome. By contrast, neither this dose of DOI in vehicle-pretreated animals, nor HU-210 treatment alone had an effect on mortality rates. While we could not examine the time-course of the hyperthermic effect of DOI (due to a loss of subjects), body temperatures exceeded 43 °C in subjects that subsequently died, suggesting that hyperthermia from DOI was enhanced following HU-210 treatment. Interestingly, the adrenocortical response elicited by DOI was unchanged following chronic HU-210 administration, suggesting that cannabinoid-induced changes in 5-HT_{2A} receptor activity may be region specific. However, the possibility also exists that the adrenocortical response from DOI had reached a ceiling effect in the vehicle-pretreated animals and thus, we were unable to detect an increase beyond that value.

The ability of DOI to induce wet-dog shakes has been linked to several neuroanatomical regions, such
as the nucleus raphe obscurus/inferior olive (Watson and Gorzalka, 1992), spinal cord (Fone et al., 1991) and the medial prefrontal cortex (Willins and Meltzer, 1997). While it has been demonstrated that wet-dog shakes induced by the 5-HT precursor, 5-HTP, remain intact following ablation of the frontal cortex (Lucki and minugh-Purvis, 1987), this alone does not demonstrate that changes in cortical levels of 5-HT$_A$ receptors would not be capable of modulating DOI-induced wet-dog shakes. Both chronic stress and glucocorticoid treatment enhance DOI-induced wet-dog shakes (Gorzalka and Hanson, 1998; Takao et al., 1997), but appear to exclusively up-regulate 5-HT$_A$ receptors in the cortex (Fernandes et al., 1997; Kuroda et al., 1992). Alternatively, chronic antidepressant administration selectively down-regulates 5-HT$_A$ receptors in the cortex while concurrently reducing DOI-induced wet-dog shakes (Goodwin et al., 1984; Metz and Heal, 1986). This suggests a strong correlation between cortical 5-HT$_A$ receptors and wet-dog shakes (Willins and Meltzer, 1997), and the possibility that chronic cannabinoïd treatment may up-regulate 5-HT$_A$ receptors in the cortex. The hypothesis that this enhancement of DOI-induced wet-dog shakes may be due to up-regulation of 5-HT$_A$ receptors in the frontal cortex, rather than in the spine is supported by the evidence that DOI-induced back muscle contractions, which are mediated by spinal 5-HT$_A$ receptors (Fone et al., 1991), are down-regulated by chronic treatment with HU-210. Additionally, the adrenergic response to DOI was unchanged, which suggests that hypothalamic 5-HT$_A$ receptors remain unchanged following this treatment; however, this cannot explain the apparent changes seen in hyperthermia. Ultimately, quantitative autoradiography would have to be performed to gain an exact understanding of the region-specific (and possibly hypothalamic nuclei-specific) changes in 5-HT$_A$ receptor levels following chronic cannabinoïd treatment.

The hypothermic response to administration of the 5-HT$_A$ receptor agonist 8-OH-DPAT was significantly attenuated at all time-points measured (30-, 60- and 90-min post-injection) in animals that had been pretreated with chronic HU-210. Moreover, the adrenergic response elicited by 8-OH-DPAT was completely attenuated following chronic HU-210 treatment. These two parameters have been proposed to represent activation of somatodendritic 5-HT$_A$ receptors in the dorsal raphe which regulate 5-HT neuronal firing and post-synaptic 5-HT$_A$ receptors in limbic regions such as the hippocampus and hypothalamus respectively (Lesch, 1991). Thus, these data suggest that prolonged cannabinoïd administration results in a decrease in both pre- and post-synaptic 5-HT$_A$ receptor activity. This could reflect a general down-regulation of the 5-HT$_A$ receptor throughout the brain, or it could represent a decrement in the signalling cascade initiated by the 5-HT$_A$ receptor, possibly due to alterations in G-protein subunit expression or coupling capacity. The exact nature of this desensitization requires further investigation.

This imbalance of 5-HT receptor responsiveness is reminiscent of changes that are seen following chronic stress or glucocorticoid administration (Bagdy et al., 1989; Berendsen et al., 1996; Gorzalka and Hanson, 1998; Gorzalka et al., 1998; Takao et al., 1997). Glucocorticoids may be one of several mediating factors in the cannabinoïdergic modulation of 5-HT receptor balance, as administration of CB$_1$ receptor agonists at doses comparable to those used in this study, elicit a robust adrenocortical response (Rodrigue de Fonseca et al., 1996, 1997). There is controversy over whether tolerance develops to this effect as some research has shown that corticosterone levels are not necessarily elevated following successive administration of a CB$_1$ receptor agonist (Pertwee, 1974); however, several studies have also demonstrated that even after multiple administrations, CB$_1$ receptor agonists do elicit a significant elevation in glucocorticoids (Collu, 1976; Miczek and Dixit, 1980; Wirguin et al., 1994). The data from this study demonstrate that basal corticosterone levels are not altered following long-term cannabinoïd treatment; however, we did not assess if corticosterone levels increased immediately following the last HU-210 injection. In any event it is unlikely that corticosterone is the only factor mediating the changes documented in this study. While long-term glucocorticoid treatment sensitizes DOI-induced wet-dog shakes and desensitizes 8-OH-DPAT-mediated hypothermia, no changes in the lethality of DOI have ever been documented following this treatment (Gorzalka and Hanson, 1998; Gorzalka et al., 1998; Matuszewich and Yamamoto, 2003; Takao et al., 1997). However, it should be noted that 10-d treatment with corticosterone has been documented to enhance the induction of 5-HT syndrome in response to carbidopa and 5-hydroxytryptophan, demonstrating that glucocorticoids do possess the ability to sensitize the induction of 5-HT syndrome (Young et al., 1992). Ultimately, the possibility exists that in addition to altering either receptor level or sensitivity, prolonged cannabinoïd treatment probably modifies other parameters of the 5-HT system. For example, if levels of 5-HT transporter were down-regulated by chronic cannabinoïd treatment, this would result in a basal elevation of synaptic
5-HT levels, which in conjunction with DOI administration, could lead to the fatal serotonin syndrome documented here. This hypothesis requires further investigation. Alternatively, prolonged cannabinoid treatment may alter tryptophan hydroxylase activity or monoamine oxidase activity, or other enzymes involved in the synthesis or metabolism of 5-HT. However, given that CB1 receptors themselves are located in brain regions implicated in thermoregulation, adrenocortical activation and serotonin syndrome, such as the hypothalamus, cortex and raphe nuclei (Herkenham et al., 1991), the possibility also exists that any changes in 5-HT receptor expression or activity could be due to direct actions of the CB1 receptor.

Due to the reciprocal nature of these receptors, one cannot rule out the possibility that changes in 5-HT2A receptor activity drove changes in 5-HT1A receptor activity, or vice versa. This is supported by evidence that 5-HT1A receptors exert negative regulation over 5-HT2A receptors (Ashby et al., 1994; Darmani et al., 1990; Williams and Meltzer, 1997). Thus, long-term cannabinoid treatment might result in a shift in the balance of 5-HT receptor activity, such that a net enhancement of 5-HT2A receptor-mediated activity predominates, perhaps via a decrement in 5-HT1A receptor activity.

Clinically, these findings may have relevance as depression is often thought of as a disorder of the 5-HT system. While there is no conclusive evidence that 5-HT levels per se are disturbed in depression, several studies suggest that 5-HT2A receptors are up-regulated and 5-HT1A receptors are down-regulated in depression and suicide (Bhagwagar et al., 2004; Drevets et al., 1999; Hrdina et al., 1993; Mann et al., 1989; Sargent et al., 2000). Moreover, there is a growing body of clinical evidence suggesting that long-term cannabis use may be associated with the development of depressive symptoms and suicide (Bovasso, 2001; Degenhardt et al., 2003; Lynskey et al., 2004). Our data provide a potential neurochemical explanation for the association between long-term cannabis use and the induction of depression. The data presented here demonstrate that long-term administration of the potent cannabinoid HU-210 results in an apparent up-regulation of 5-HT2A receptor activity and a down-regulation of 5-HT1A receptor activity. Furthermore, the blunted adrenocortical and hypothermic response to administration of 8-OH-DPAT found following long-term cannabinoid treatment in this study are very reminiscent of the attenuated hormonal and hypothermic responses to 5-HT1A receptor agonists in depressed individuals (Cowen et al., 1994; Lesch et al., 1990a–c; Meltzer and Maes, 1995; Shapira et al., 2000). These are the first data to demonstrate that chronic cannabinoid treatment can modify the 5-HT system and may aid in understanding the effects long-term cannabis use has on affective behaviour and disease.

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Statement of Interest

None.

References


Bedard P, Pycock CJ (1977). ‘Wet-dog’ shake behaviour in the rat: a possible quantitative model of central...


Fone KC, Johnson JV, Bennett GW, Marsden CA (1989). Involvement of 5-HT2 receptors in the behaviours produced by intrathecal administration of selected 5-HT agonists and the TRH analogue (CG 3509) to rats. *British Journal of Pharmacology* 96, 599–608.


Metz A, Heal DJ (1986). In mice repeated administration of electroconvulsive shock or desmethylimipramine produces rapid alterations in 5-HT2-mediated head-twitch responses and cortical 5-HT2 receptor number. European Journal of Pharmacology 126, 159–162.


