Published in final edited form as:

Addict Biol. 2013 November ; 18(6): . doi:10.1111/j.1369-1600.2011.00369.x.

Cannabinoid-1 receptor antagonist rimonabant (SR141716) increases striatal dopamine D2 receptor availability

Cleo L. Crunelle^{*,1,2}, Elsmarieke van de Giessen^{*,2}, Sybille Schulz³, Louk J.M.J. Vanderschuren^{4,5}, Kora de Bruin², Wim van den Brink¹, and Jan Booij²

¹Amsterdam Institute for Addiction Research and Department of Psychiatry, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands ²Department of Nuclear Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

³Department of Neuropharmacology, Brain Research Institute, University of Bremen, Germany

⁴Department of Neuroscience and Pharmacology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands ⁵Department of Animals in Science and Society, Division of Behavioural Neuroscience, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Abstract

The cannabinoid 1 (CB₁) receptor antagonist rimonabant (SR141716) alters rewarding properties and intake of food and drugs. Additionally, striatal dopamine D2 receptor (DRD2) availability has been implicated in reward function. This study shows that chronic treatment of rats with rimonabant (1.0 and 3.0 mg/kg/day) dose-dependently increased DRD2 availability in the dorsal striatum (14% and 23%) compared to vehicle. High-dose rimonabant also increased DRD2 availability in the ventral striatum (12%) and reduced weight gain. Thus, upregulation of striatal DRD2 by chronic rimonabant administration may be an underlying mechanism of action and confirms the interactions of the endocannabinoid and dopaminergic systems.

Keywords

cannabinoid 1 receptor; dopamine D2 receptor; IBZM; nucleus accumbens; rimonabant; striatum

The cannabinoid 1 (CB₁) receptor antagonist/reverse agonist rimonabant (SR141716) was developed for the treatment of obesity. It has shown to decrease intake of and responding towards food and drug stimuli, which has been related to its effect on reward processing (for review: Solinas *et al* 2008). Indeed, the mesolimbic dopaminergic (DAergic) reward system and the cannabinoid system are strongly interconnected (Solinas *et al* 2008). Preclinical and clinical research have shown that striatal DA D₂ receptor (DRD2) availability is decreased in obesity (Johnson and Kenny, 2010; Wang *et al* 2001) and drug dependence (Nader *et al* 2006; Volkow *et al* 2004), which has led to the hypothesis of reward deficiency in obesity and drug addiction. An increase in DRD2 availability might therefore be beneficial in these disorders (Nader *et al* 2006). Interestingly, knock-out mice for the CB₁ receptor have an

Corresponding author: Cleo L. Crunelle, Department of Psychiatry, room PA3-229, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, Tel: 0031-20-5668322, c.l.crunelle@amc.uva.nl.

*Both authors contributed equally

Author contribution

The study was initiated and conducted by academic investigators at the Academic Medical Center of the University of Amsterdam.

All authors declare no conflict of interest.

increased number of DRD2 (Houchi *et al* 2005) and acute treatment with rimonabant reduces DA release in the nucleus accumbens (NAcc) after food or drug intake (Melis *et al* 2007; Cohen *et al* 2002). However, the effects of sustained rimonabant treatment on the striatal DAergic system have not yet been studied. Therefore, we tested our hypothesis that sustained rimonabant treatment may lead to an increase of striatal DRD2 availability.

Adult male Wistar rats (Harlan, Horst, The Netherlands) were housed in a temperature and humidity controlled room with a 12 hours light/dark cycle (lights on 7:00 am – 7:00 pm) with food and water ad libitum. All procedures were approved by the Animal Ethics Committee (AMC, Amsterdam, The Netherlands) and conducted in agreement with European regulations (Guideline 86/609/EEC). Following a 7-day habituation period, animals were randomized to daily intraperitoneal (i.p.) injections of either vehicle (n = 16), 1.0 mg/kg rimonabant (n = 16), or 3.0 mg/kg rimonabant (n = 16) for 13 consecutive days. Rimonabant (SR141716) was kindly provided by the National Institute of Mental Health Chemical Synthesis and Drug Supply Program (NIMH: Bethesda, MD, USA). It was dissolved daily prior to usage in ethanol (96% EtOH), Tween-80, and saline (0.9% NaCl) in a 1:1:18 ratio, which was also used as vehicle. Body weight was measured on days 1, 3, 6, 8, 11, and 14. On day 14, rats were anesthetized with ketamine-xylazine (ratio 2:1) and intravenously injected with approximately 37 MBq ^{123}I -IBZM (GE Healthcare, Eindhoven, The Netherlands), a selective and well-validated DRD2 tracer (binds to both DA D2 and D3 receptors). Rats were sacrificed 90 minutes following ^{123}I -IBZM injection by bleeding via heart puncture and brains were removed. Next, storage phosphor imaging was used, as described previously (Crunelle *et al* 2009), to determine DRD2 availability as ratios of ventral or dorsal striatum-to-cerebellum binding (Figure 2A).

Statistical analyses were performed with SPSS 16.0 (SPSS inc. Chicago, IL, USA) using ANOVAs with Tukey post-hoc tests for normally distributed weight data (one-way for body weight at start; repeated-measures for weight gain) and Kruskal-Wallis with post-hoc Mann-Whitney U tests for non-parametric DRD2 availability data. A probability value of $p < 0.05$ was considered significant.

Six animals were excluded from analyses due to death during anaesthesia (2) or data acquisition failure (4), leaving group sizes of 12 for vehicle, 14 for rimonabant 1.0 mg/kg, and 16 for rimonabant 3.0 mg/kg. Body weights did not differ between groups at treatment start on day 1 (vehicle (mean $\pm$ standard deviation): 296.8 ± 19.0 g, rimonabant 1.0 mg/kg: 293.5 ± 13.1 g, rimonabant 3.0 mg/kg: 298.5 ± 19.6 g). However, weight gain during treatment differed between groups ($F = 7.35$, $p = 0.002$), as the 3.0 mg/kg rimonabant treated animals gained significantly less weight (38.9 ± 9.8 g) than the vehicle group (52.3 ± 12.3 g; $p = 0.002$) and the rimonabant 1.0 mg/kg group (49.4 ± 13.9 g; $p = 0.030$) (Figure 1). DRD2 availability in the dorsal striatum differed between groups ($H = 15.75$, $p < 0.001$; Figure 2B) with significantly higher availability in the 3.0 mg/kg rimonabant group (median: 5.31, interquartile range (iqr): 0.55) compared to vehicle (median: 4.32, iqr: 0.41, $Z = -3.34$, $p = 0.001$, difference 23%) and to the 1.0 mg/kg rimonabant group (median: 4.91, iqr: 0.45, $Z = -2.41$, $p = 0.016$). Moreover, the 1.0 mg/kg rimonabant group had significantly higher DRD2 availability in the dorsal striatum compared to vehicle ($Z = -2.75$, $p = 0.006$, difference 14%). In the ventral striatum, there was a trend for different DRD2 availability between groups ($H = 5.37$, $p = 0.068$, Figure 2C) with post-hoc tests revealing that DRD2 availability of the 3.0 mg/kg rimonabant group (median: 3.22, iqr: 0.61) was 12% higher compared to vehicle (median: 2.87, iqr: 0.55, $Z = -2.41$, $p = 0.016$). Ventral striatal DRD2 availability for the 1.0 mg/kg group (median: 3.21, iqr: 0.48) was not different from the other groups.

In summary, administration of rimonabant for 13 consecutive days dose-dependently increased DRD2 availability in the dorsal striatum and high-dose rimonabant (3.0 mg/kg/day) increased DRD2 availability in the ventral striatum. Additionally, weight gain during the experiment was significantly decreased in the 3.0 mg/kg/day group compared to vehicle, which is in line with the well-known effect of rimonabant to reduce body weight.

The increase of striatal DRD2 availability forms possibly part of the underlying mechanism of action of rimonabant to normalize aberrant reward-related behavior. Similarly, CB₁ receptor knockout mice display reduced ethanol-induced conditioned place preference in combination with increased striatal DRD2 (Houchi *et al* 2005). It has indeed been hypothesized that upregulation of striatal DRD2 could reduce drug administration (Nader *et al* 2006). Interestingly, DRD2 availability was increased in the ventral striatum, which plays a primary role in motivation and reward. However, the effect of rimonabant on DRD2 availability was largest in the dorsal striatum. This region has been implicated in different forms of instrumental responding for food and habit formation (Yin *et al* 2008). The latter is impaired by CB₁ receptor blockade (Hilario *et al* 2007) suggesting that rimonabant leads to less habitual food intake, which might be related to increased DRD2 availability in the dorsal striatum. Alternatively, it may be that the effects of rimonabant are larger on DRD2 than on DRD3, since the ratio of DRD2 versus DRD3 is larger in dorsal than in ventral striatum.

The underlying mechanism of the reported effect is possibly mediated via antagonism of CB₁ receptors in the ventral tegmental area and substantia nigra. They were found to modulate indirectly, i.e. via glutamatergic axons, and possibly also directly the inhibitory gamma aminobutyric acid (GABA)ergic inputs to DAergic neurons that project to the striatum, therewith leading to lower levels of extracellular DA (Lupica and Riegel 2005; Schlicker and Kathmann 2001). This could result in a subsequent upregulation of striatal DRD2. This proposed mechanism is supported by the ability of acute rimonabant administration to decrease the DA release in the NAcc by food or drug intake, therewith reducing the rewarding effect of food/drugs (Melis *et al* 2007; Cohen *et al* 2002).

A limitation of the study is that the thirteen days of treatment may not have been long enough to detect a significant increase of DRD2 availability in the ventral striatum for the 1.0 mg/kg/day dose. The results for the dorsal striatum suggest a dose-dependent effect of rimonabant on DRD2 availability, though. Future studies should consider different dosages and treatment durations and administration of rimonabant in the active 'dark' cycle for possible larger effects. In addition, the hour of sacrifice of the rats might be important, as it has been shown that endocannabinoid and CB₁ receptor levels in the rat brain are modulated by the light/dark cycle (Martinez-Vargas *et al* 2003; Valenti *et al* 2004). Further, this study does not provide direct evidence that increased DRD2 availability is related to reduced food intake. We can also not exclude that reduced caloric intake by rimonabant administration has had a direct effect on DRD2 availability. Future studies should consider measuring locomotor activity and food intake for a correlation with (changes in) striatal DRD2 availability, and might introduce a group with food restriction as a control. Finally, a systematic effect of the anaesthetic ketamine on DRD2 availability can not be excluded.

In conclusion, we found that chronic treatment with rimonabant significantly increased DRD2 receptor availability in dorsal and ventral striatum of rats. It demonstrates how the endocannabinoid system interacts with the striatal DAergic system and supports its important role in reward-related disorders. Although rimonabant has been removed from the market due to psychiatric side effects, insight in the mechanisms of the cannabinoid system may yield new implications for the pharmacological treatment of individuals with eating disorders, drug abuse, and other reward-deficiency disorders.

Acknowledgments

Financial support was provided by the Academic Medical Center and ZonMW/NIDA (nr 311-80-003).

References

- Cohen C, Perrault G, Voltz C, Steinberg R, Soubrié P. SR141716, a central cannabinoid (CB₁) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav Pharmacol.* 2002; 13:451–463. [PubMed: 12394421]
- Crunelle CL, Miller ML, de Bruin K, van den Brink W, Booij J. Varenicline increases striatal dopamine D_{2/3} receptor binding in rats. *Addict Biol.* 2009; 14:500–502. [PubMed: 19650815]
- Hilario MR, Clouse E, Yin HH, Costa RM. Endocannabinoid signaling is critical for habit formation. *Front Integr Neurosci.* 2007; 1:6. [PubMed: 18958234]
- Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, Naassila M. CB1 receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. *Neuropsychopharmacol.* 2005; 30:339–349.
- Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci.* 2010; 13:635–641. [PubMed: 20348917]
- Lupica CR, Riegel AC. Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. *Neuropharmacol.* 2005; 48:1105–1116.
- Martinez-Vargas M, Murillo-Rodriguez E, Gonzalez-Rivera R, Landa A, Mendez-Diaz M, Prospero-Garcia O, et al. Sleep modulates cannabinoid receptor 1 expression in the pons of rats. *Neurosci.* 2003; 117:197–201.
- Melis T, Saccu S, Sanna F, Boi A, Argiolas A, Melis MR. The cannabinoid antagonist SR 141716A (Rimonabant) reduces the increase of extra-cellular dopamine release in the rat nucleus accumbens induced by a novel high palatable food. *Neurosci Lett.* 2007; 419:231–235. [PubMed: 17462824]
- Nader MA, Morgan D, Gage HD, Nader SH, Calhoun TL, Buchheimer N, et al. PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. *Nat Neurosci.* 2006; 9:1050–1056. [PubMed: 16829955]
- Schlicker E, Kathmann M. Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci.* 2001; 22:565–572. [PubMed: 11698100]
- Solinas M, Goldberg SR, Piomelli D. The endocannabinoid system in brain reward processes. *Br J Pharmacol.* 2008; 154:369–383. [PubMed: 18414385]
- Valenti M, Vigano D, Cascio MG, Rubino T, Steardo L, Parolaro D, et al. Differential diurnal variations of anandamide and 2-arachidonoyl-glycerol levels in rat brain. *Cell Mol Life Sci.* 2004; 61:945–950. [PubMed: 15095014]
- Volkow ND, Fowler JS, Wang GJ, Swanson JM. Dopamine in drug abuse and addiction: results from imaging studies and treatment implications. *Mol Psychiatry.* 2004; 9:557–569. [PubMed: 15098002]
- Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, et al. Brain dopamine and obesity. *Lancet.* 2001; 357:354–357. [PubMed: 11210998]
- Yin HH, Ostlund SB, Balleine BW. Reward-guided learning beyond dopamine in the nucleus accumbens: the integrative functions of cortico-basal ganglia networks. *Eur J Neurosci.* 2008; 28:1437–1448. [PubMed: 18793321]

Figure 1.
Cumulative weight gain curves. Means + s.e.m.

Figure 2.

A. Examples of regions of interest for dorsal striatum (A) and ventral striatum (B), and cerebellum (C).

B. DRD2 availability in dorsal striatum following rimonabant treatment. Median + interquartile range. * $p < 0.05$, ** $p < 0.01$.

C. DRD2 availability in ventral striatum following rimonabant treatment. Median + interquartile range. * $p < 0.05$, ** $p < 0.01$.