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

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ABSTRACT

The cannabinoid 1 receptor antagonist rimonabant (SR141716) alters rewarding properties and intake of food and drugs. Additionally, striatal dopamine D_2 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 with vehicle. High-dose rimonabant also increased DRD2 availability in the ventral striatum (12%) and reduced weight gain. Thus, up-regulation 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.

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The cannabinoid 1 (CB_1) 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, see Solinas, Goldberg & Piomelli 2008). Indeed, the mesolimbic dopaminergic (DAergic) reward system and the cannabinoid system are strongly interconnected (Solinas *et al.* 2008). Pre-clinical and clinical research have shown that striatal dopamine (DA) D_2 receptor (DRD2) availability is decreased in obesity (Johnson & Kenny 2010) and drug dependence (Nader *et al.* 2006), 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, knockout mice for the CB_1 receptor have an 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 (Cohen *et al.* 2002; Melis *et al.* 2007). 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.
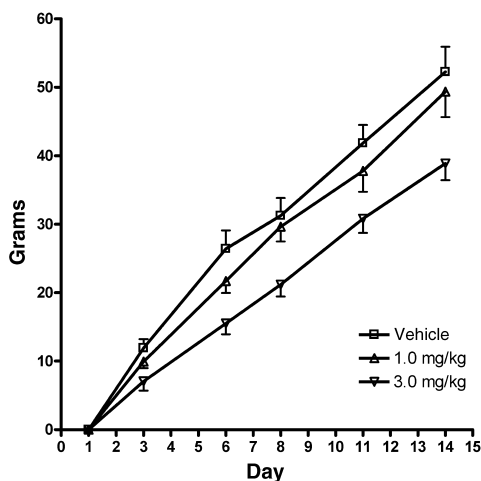
Adult male Wistar rats (Harlan, Horst, the Netherlands) were housed in a temperature- and humidity-controlled room with a 12-hour light/dark cycle (lights on 7:00 AM–7:00 PM) with food and water *ad libitum*. All procedures were approved by the Animal Ethics Committee (AMC, Amsterdam, the Netherlands) and conducted in agreement with European regulations (Guideline 86/609/EEC). Following a 7-day habituation period, animals were randomized to daily intraperitoneal injections of either vehicle ($n = 16$), 1.0 mg/kg rimonabant ($n = 16$) or 3.0 mg/kg rimonabant ($n = 16$) for 13

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consecutive days. Rimonabant (SR141716) was kindly provided by the National Institute of Mental Health Chemical Synthesis and Drug Supply Program (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-iodobenzamide (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 their 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 (Fig. 2a).

Statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA) using analyses of variance with Tukey's *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 (two) or data acquisition failure (four), 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 ± 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; $P = 0.030$) (Fig. 1).

DRD2 availability in the dorsal striatum differed between groups ($H = 15.75$, $P < 0.001$; Fig. 2b) with significantly higher availability in the 3.0 mg/kg rimonabant group (median: 5.31, interquartile range (iqr): 0.55) compared with the vehicle (median: 4.32, iqr:

Figure 1 Cumulative weight gain curves. Means + standard error of the mean
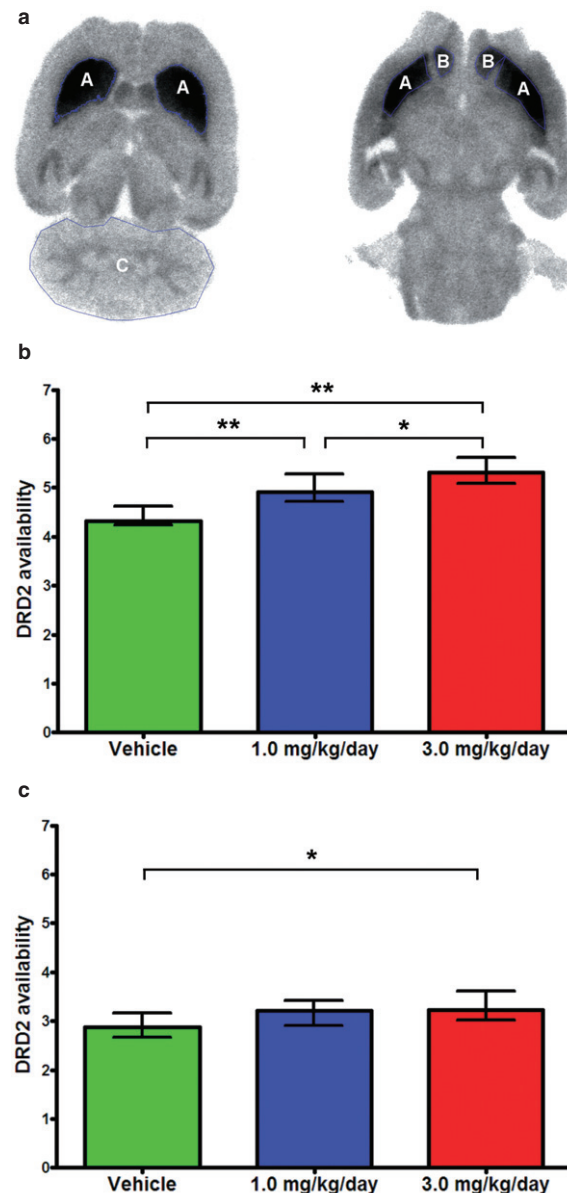
Figure 2 (a) Examples of regions of interest for dorsal striatum (A) and ventral striatum (B), and cerebellum (C). (b) D2 receptor (DRD2) availability in dorsal striatum following rimonabant treatment. Median + interquartile range. *$P < 0.05$, **$P < 0.01$. (c) DRD2 availability in ventral striatum following rimonabant treatment. Median + interquartile range. *$P < 0.05$, **$P < 0.01$

0.41, Z = −3.34, P = 0.001, difference 23%) and with 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 with 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, Fig. 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 with the 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 with 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 behaviour. Similarly, CB_1 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 up-regulation 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. The latter is impaired by CB_1 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, because 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 CB_1 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 aminobutergic acid (GABA)ergic inputs to DAergic neurons that project to the striatum, therewith leading to lower levels of extracellular DA (Lupica & Riegel 2005). This could result in a subsequent up-regulation of striatal DRD2. This proposed mechanism is supported by the ability of acute rimonabant administration to decrease DA release in the NAcc by food or drug intake, therewith reducing the rewarding effect of food/drugs (Cohen *et al.* 2002; Melis *et al.* 2007).

A limitation of the study is that the 13 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 CB_1 receptor levels in the rat brain are modulated by the light/dark cycle (Martinez-Vargas *et al.* 2003). 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 cannot 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 because of 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.

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Authors 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. All authors have critically reviewed content and approved final version submitted for publication.

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