Development of new neurobiological strategies to treat patients with cocaine dependence
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Chapter 6:

Cannabinoid-1 receptor antagonist rimonabant (SR141716) increases striatal dopamine D$_{2/3}$ receptor availability

From:
The cannabinoid 1 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 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.
Introduction

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, 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) D2 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 CB1 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.

Materials and Methods

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 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 DRD$_2$ tracer (binds to both DA D$_2$ and D$_3$ 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 DRD$_2$ 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 bodyweight at start; repeated measures for weight gain) and Kruskal–Wallis with post hoc Mann–Whitney U-tests for non-parametric DRD$_2$ availability data. A probability value of $P < 0.05$ was considered significant.

**Results**

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).

DRD$_2$ 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: 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
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**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) D$_2$ receptor (DRD$_2$) availability in dorsal striatum following rimonabant treatment. Median ± interquartile range. *$p<0.05$, **$p<0.01$. (c) DRD$_2$ availability in ventral striatum following rimonabant treatment. Median ± interquartile range. *$p<0.05$, **$p<0.01$. 
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.

**Discussion and conclusions**

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, 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 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 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, 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 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 aminobuteric 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 CB1 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.