Development of new neurobiological strategies to treat patients with cocaine dependence

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Chapter 4

Dose-dependent and sustained effects of varenicline on dopamine $D_{2/3}$ receptor availability in rats

From:
Abstract

Imaging studies in drug-dependent subjects show reduced striatal dopamine D2/3 receptor (DRD2/3) availability, and it is hypothesized that increasing DRD2/3 availability is a promising strategy to treat drug dependence. We recently showed that rats treated for two weeks with 2mg/kg/day varenicline (a partial agonist at α4β2 nicotinic acetylcholine receptors) showed higher striatal DRD2/3 availability compared to control rats. The present study examined the effects of lower varenicline doses as well as the duration of the effect after treatment discontinuation. DRD2/3 availability in striatal areas was studied in 80 rats following two-week treatment with 0.5, 1 or 2mg/kg/day varenicline or vehicle and survival of the effects of varenicline on DRD2/3 availability up to 2 weeks after treatment discontinuation using [123I]IBZM storage phosphor imaging. For all varenicline doses, varenicline treated rats showed a comparable significantly higher DRD2/3 availability in the ventral striatum of approximately 11% compared to control rats, while only the rats treated with 1 and 2mg/kg/day dose showed significantly higher DRD2/3 availability in the dorsal striatum by 12.5% and 13.2% compared to control rats, respectively. Two weeks after discontinuation of the active treatment with 2mg/kg/day varenicline, DRD2/3 binding in ventral, but not dorsal, striatum was still significantly higher (11.7%) compared to vehicle. Varenicline induces dose-dependent and sustained increases in striatal DRD2/3 in rats, particularly in the ventral striatum. These observations suggest that increased DRD2/3 availability may contribute to varenicline’s efficacy for smoking cessation and show promise for varenicline as a treatment of other types of drug dependence.
1. Introduction

A consistent finding in human imaging studies in drug dependence, including nicotine dependence, is a lower receptor density of striatal dopamine (DA) D_{2/3} receptors (DRD_{2/3}) (Beukers et al., 2009; Fehr et al., 2008; Martinez et al., 2004). For example, in cocaine-dependent patients, Martinez et al. found a 15% reduction in striatal DRD_{2/3} availability compared to healthy subjects (Martinez et al., 2004), a finding that was replicated in another cohort (Martinez et al., 2009). In addition, acute dopaminergic depletion induced by alpha-methyl-paratyrosine (AMPT) administration showed lower occupancy of striatal DRD_{2/3} by endogenous dopamine. Therefore, reduction in DRD_{2/3} could not be attributed to higher levels of endogenous DA in the striatal regions of cocaine-dependent patients during PET imaging (Martinez et al., 2009).

Varenicline is a registered aid for smoking cessation and clinically results in reduced pleasure while smoking, decreased craving, and an increased probability of long-term abstinence (Cahill et al., 2008). Varenicline is a high affinity partial agonist at $\alpha_4\beta_2$ nicotinic acetylcholine receptors (nAChR) with a much lower binding affinity for other nAChR subtypes at which it either acts as a partial agonist (e.g. $\alpha_3\beta_4$) or as a full agonist ($\alpha_7$) (Mihalak et al., 2006; Rollema et al., 2007, 2010). Interestingly, preclinical studies have shown that varenicline indirectly interacts with the DAergic system and seems to result in increased DRD_{2/3} availability in the striatum (for a review, see Crunelle et al., 2010). Indeed, about one third of $\alpha_4\beta_2$ nAChRs are localized on DAergic cell bodies of the mesolimbic DA reward circuitry (Zhou et al., 2003) and ($\beta_2$-containing) nAChRs are believed to modulate and enhance DA cell firing in the ventral tegmental area (VTA), characteristic for reward-related signaling (Dani, 2001; Reperant et al., 2010; Rice and Cragg, 2004; Tang and Dani, 2009). Moreover, in a previous study we observed that rats treated with varenicline (2 mg/kg/day injected for 14 consecutive days) showed a significantly higher striatal DRD_{2/3} availability than control rats (Crunelle et al., 2009). However, this experiment had several limitations: it was performed with one relatively high dose of varenicline and did not examine the sustained effects of varenicline on DRD_{2/3} availability after discontinuation of treatment. Together, these findings raise the possibility that varenicline could also be an effective treatment for addictions other than nicotine dependence (Chatterjee and Barlett, 2010; Crunelle et al., 2010; McKee et al., 2010), because previous studies have
suggested that upregulation of the DRD2/3 is a promising strategy for the treatment of addiction in general (Nader et al., 2006; Blum et al., 2008).

In the current study, we examined the effects of two week treatment with three doses of varenicline on DRD2/3 availability in striatal regions of rats and whether the effect of two-week treatment with the highest dose of varenicline on DRD2/3 availability was sustained in striatal regions up to two weeks after the last injection with varenicline.

2. Experimental procedures

2.1. Animals

Eighty male Wistar rats (Harlan, The Netherlands), weighing 250 - 300 g at the start of the experiment, were housed in groups of five in a temperature- and humidity-controlled room under a 12/12 h light cycle (lights on at 07:00 am). Rats were given one week to acclimatize before the experiments. Food and water were available ad libitum and body weight was recorded daily throughout the experiment. All experimental procedures were approved by the Animal Ethics Committee (AMC, Amsterdam, the Netherlands).

2.2. Dosing regimen

To assess the effects of several doses of varenicline on DRD2/3 availability, rats were randomized to varenicline or vehicle (0.9% saline). Varenicline was administered subcutaneously (s.c.) twice daily (BID), because of its short half-life of 4 h in rats (Obach et al., 2006), at 8:00–9:00 am and 3:00–4:00 pm. Control rats (n=10) were administered vehicle for 14 consecutive days, at times similar to those of the varenicline administrations, twice per day. One day after the last injection, ex-vivo measurement of DRD2/3 availability was performed using storage phosphor imaging (Knol et al., 2008; Crunelle et al., 2009).

The sustained effects of varenicline on DRD2/3 availability after treatment discontinuation were studied in rats that were randomized to either varenicline 2 mg/kg/day BID or vehicle BID for 14 consecutive days. DRD2/3 availability was assessed using ex-vivo storage phosphor imaging at one week and at two weeks after the last injection with either varenicline or vehicle (n= 10 and n=10).
2.3. Ex-vivo storage phosphor imaging

The imaging method used to assess DRD<sub>2/3</sub> availability has been described in detail before (Crunelle et al, 2009; Knol et al, 2008). Briefly, rats were anesthetized and 0.20 ml ± 0.05 ml of the selective DRD<sub>2/3</sub> radiotracer [123I]IBZM (37.7 MBq ± 2.8 MBq) was given i.v. through the tail vein. Ninety minutes after IBZM injection (Verhoeff et al, 1999), brains were removed and sliced into 50 μm slices. Every fourth brain slice was stored on phosphor imaging plates for 16 h ± 1 h. Regions of interest (ROIs) were drawn around the left and right striatum and the cerebellum (devoid of DRD<sub>2/3</sub>) for each rat according to a standard rat brain atlas (Paxinos and Watson, 1986), as earlier described (Crunelle et al, 2009). Subsequently, IBZM binding in ROIs (expressed as photostimulated luminescence) was analyzed using AIDA imaging software.

For both right and left dorsal striatum, 13 consecutive slices with highest binding were selected for each rat. For the ventral striatum (nucleus accumbens), two consecutive slices with the highest binding for both left and right hemispheres were selected. Non-specific binding was determined by selecting nine consecutive slices of the cerebellum with the highest binding. Ratios of striatal-to-cerebellar binding were then calculated using the following equation: [(left + right) striatum / 2] / cerebellum. These ratios were interpreted as a measure for DRD<sub>2/3</sub> availability.

2.4. Study medication

Varenicline (7,8,9,10-tetrahydro- 6,10-methano-6H-pyrazino (2,3-h) (3)-benzazepine) tartrate was kindly provided by Pfizer Global Research and Development (Groton, CT, USA), and dissolved in 0.9% sodium chloride to obtain an injectable solution. In all experiments doses are expressed as the active compound (base). Solutions were made fresh twice a week, and were kept refrigerated during the experiment. The radiotracer [123I]IBZM was commercially produced by GE Healthcare (Eindhoven, The Netherlands) with a specific activity of 550 MBq/mol and a radiochemical purity of >95%. Anesthesia was performed with a 2:1 mix of ketamine and xylazine.

2.5. Statistical analyses

Data were analyzed with SPSS 17 (SPSS Inc, Chicago, IL, USA), using a probability value of 0.05 as significant. All data were normally distributed and a one-way ANOVA was used to assess between-group differences. Post-hoc analyses were performed using independent sample T-tests for non-repeated measures and assuming equal variances. To assess differences between right and left striatum, a dependent-sample T-test was used.
3. Results

Data from 4 rats were excluded from the analyses due to premature death following anesthesia and [123I]IBZM injection (2 rats) or due to data acquisition failures (2 rats).

Since there were no differences between binding ratios in left and right striatum (paired differences ± SD: 0.01 ± 0.09; \( p = 0.79 \) for the dorsal striatum, and 0.03 ± 0.09; \( p = 0.43 \) for the ventral striatum) all data are presented as the mean binding ratio of left and right sides. Furthermore, all data were normally distributed (Shapiro–Wilk test; \( p = 0.60 – 0.90 \)).

3.1. Experiment 1: Effect of several doses of varenicline on DRD\(_{2/3}\) availability

Rats treated with the lowest dose of varenicline (0.5 mg/kg/day; \( n = 9 \)) showed a significant 11.1% higher binding ratio in the ventral striatum compared to vehicle-treated rats (\( p = 0.015; 95\% \text{ CI} = 2.5\%–19.7\%, n = 9 \)), but no statistically significant difference in binding ratios in the dorsal striatum (\( p = 0.590; \text{NS} \)) was found (Fig. 1). At 1 mg/kg/day, rats treated with varenicline showed a significantly higher DRD\(_{2/3}\) availability by 11.1% in the ventral striatum (\( p = 0.009; 95\% \text{ CI} = 3.1\%–19.1\%, n = 10 \)) and by 12.5% in the dorsal striatum (\( p = 0.048; 95\% \text{ CI} = 0.2\%–24.9\%) compared to vehicle (\( n = 9 \)) (see Fig.

![Figure 1](image-url)

**Figure 1:** Effects of 14 days s.c. varenicline treatment in rats on [123I]IBZM binding ratios in the dorsal striatum (A) and the ventral striatum (B) using ex-vivo storage phosphor imaging. Bars represent striatal-to-cerebellar binding ratios expressed as mean ± SD. *\( p < 0.05 \) vs. vehicle-treatment.
Finally, rats treated with the highest dose of varenicline (2 mg/kg/day; \(n = 10\)) showed significant higher binding ratios in both ventral striatum (11.1\% increase; \(p = 0.036, 95\% CI = 0.8\%–21.4\%) and dorsal striatum (13.2\% increase; \(p = 0.030, 95\% CI = 1.4\%–25.0\%\)) compared to vehicle-treated rats (\(n = 9\)) (see Fig. 1).

### 3.2. Experiment 2: Sustained effects of varenicline treatment on DRD_{2/3} availability

One week after discontinuation of treatment with 2 mg/kg/day varenicline for 14 days, the increase in binding ratios was still significantly higher in the ventral striatum (12.8\% increase; \(p = 0.018; 95\% CI: 2.5\%–23.1\%\), \(n = 9\)) compared to vehicle treatment one week after discontinuation of vehicle injections (\(n = 10\)), but not in the dorsal striatum (6.4\% increase, \(p = 0.287\)) (see Fig. 2). Similar changes were observed one week later, i.e. at two weeks after varenicline treatment, with statistically higher binding ratios in the ventral striatum (11.7\% increase; \(p = 0.015; 95\% CI: 2.6\%–20.8\%\), \(n = 10\)), but not in the dorsal striatum (8.2\% increase, \(p = 0.09\)) compared to vehicle treatment two weeks after discontinuation of vehicle injections (\(n = 9\)) (see Fig. 2).

![Figure 2](image-url): Effects of post-treatment time of varenicline in rats (treated for 14 days, s.c.) on \([^{123}I]\)IBZM binding ratios in the dorsal striatum (A) and ventral striatum (B) using ex-vivo storage phosphor imaging. Bars represent striatal-to-cerebellar binding ratios and are represented as mean ± SD. *\(p<0.05\) vs. vehicle.
Rats treated with varenicline 2 mg/kg/day for 14 consecutive days had significantly higher DRD$_{2/3}$ availabilities in the dorsal striatum (13.2%) and the ventral striatum (11.1%) compared to vehicle-treated rats, which is a close replication of results from a previous study using the same varenicline treatment that found higher DRD$_{2/3}$ availabilities of 14.7% and 13.9% in dorsal and ventral striatum compared to controls, respectively (Crunelle et al., 2009). The present study also examined the effects of two lower doses of varenicline and found that one day after 2-week treatment with 0.5 and 1 mg/kg/day varenicline, DRD$_{2/3}$ availability was higher in the ventral striatum by 11.1% for both doses compared to controls, and that only the 1 mg/kg/day dose showed a significantly higher DRD$_{2/3}$ availability in the dorsal striatum by 12.5% compared to controls. In addition, up to two weeks after the last injection of 14 consecutive days treatment with 2 mg/kg/day varenicline, DRD$_{2/3}$ availability was still significantly higher than controls in the ventral striatum (12.8%), but not in the dorsal striatum.

The present design and results do not allow us to advocate a specific mechanism of action by which varenicline increases striatal DRD$_{2/3}$ availability in the rat striatum. Varenicline acts at $\alpha_4\beta_2$ nAChRs and has a partial agonist effect in the absence of nicotine, reducing withdrawal during a quit attempt, and an antagonist effect when actively smoking, blocking the rewarding effects of nicotine by competing for nAChRs (Rollema et al., 2007). It is known that about one third of $\alpha_4\beta_2$ nAChRs in the brain are localized on DAergic cell bodies of the mesostriatal neurons (Zhou et al., 2003) and that $\beta_2$-containing nAChRs are believed to enhance DA cell firing and DA release (Dani, 2001; Reperant et al., 2010; Rice and Cragg, 2004; Tang and Dani, 2009). Preclinical studies have indeed shown that acute administration of both nicotine and varenicline increases DA release and turnover with a smaller maximal effect in varenicline (~150% increase) compared to nicotine (~180%) (Rollema et al., 2007; Ericson et al., 2009). Recently, it was demonstrated that this effect of varenicline, similar to nicotine-induced mesolimbic DA increases, is most likely mediated via $\alpha_4\beta_2$ nAChRs in the ventral tegmental area (VTA), which contains dopaminergic neurons that project predominantly to the ventral striatum (Reperant et al., 2010). It is, however, unlikely that varenicline-evoked DA release would induce the presently observed increase in striatal DRD$_{2/3}$ availability for a number of reasons. First, smoking a regular cigarette, which induces acute dopamine release, resulted
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in a significant decrease of striatal DRD2/3 receptors particularly in ventral parts of the striatum (Brody et al., 2006). Second, the present study showed that increase in striatal DRD2/3 availability persists up to 2 weeks after varenicline discontinuation. Third, the studies by Ericson et al. (2009) and Rollema et al. (2007) measured DA release extracellularly using in-vivo microdialysis, while ex-vivo storage phosphor imaging measures DRD2/3 binding at the synaptic level (Schiffer et al., 2006). Finally, rats treated with several doses of varenicline had similar effects on DRD2/3 binding in the dorsal and ventral striatum while it was recently demonstrated that the effects of varenicline and smoking on the dopaminergic system are most likely mediated predominantly via α4β2 nAChRs in the VTA (Brody et al., 2006; Reperant et al., 2010). Instead, it is conceivable that long-term exposure to varenicline could cause reduced functioning of the DAergic system, leading to a decrease in synaptic DA and consequently to an upregulation of striatal DRD2/3, which are located predominantly postsynaptically. Alternatively, but speculative, the increase in DRD2/3 availability after chronic varenicline treatment might be due to effects of varenicline on possible molecular pathways involved directly in the expression of DRD2/3. Like most G-protein-coupled receptors, DRD2/3 expression is regulated by kinases and arrestins (Ito et al., 1999; Kim et al., 2001). However, to the best of our knowledge, the effects of varenicline on such kinases and arrestins have not yet been studied.

We were not able to show dose-dependent effects of varenicline. This might be due to ceiling effect from the doses chosen, and therefore, experiments with doses up to 0.5 mg/kg/day should be performed to further assess dose-dependency. Additionally, in the present study, experiments were performed in rats not exposed to nicotine. Since exposure to nicotine may induce dopaminergic hypersensitivity (Novak et al., 2010), future studies on the effects of varenicline on DRD2/3 receptor availability in nicotine-dependent and nicotine-abstinent rats may be relevant to test if one may generalize our present findings.

In humans, acute administration of varenicline does not alter smoking behaviour at peak-plasma levels (Williams et al., 2007), indicating the importance of studying the effects of a longer treatment with varenicline as was done in the current experiments. The current study showed a clear sustained effect of varenicline on DRD2/3 availability in the ventral striatum with similar increases at 1 day (11%), 1 week (13%), and 2 weeks (12%) following treatment discontinuation. This observation indicates that chronic administration of varenicline may lead to upregulation of striatal DRD2/3 through neuroadaptation (vide infra).

The main strengths of the present study are that several dosages of varenicline were tested, the large variation in post-treatment duration before DRD2/3 availability as-
assessment, and the use of a well-validated assessment procedure. A limitation of the study with respect of its translational value is BID administration of varenicline that has a half-life in rats of about 4 h (Obach et al., 2006). For instance, peak levels in rat plasma after a single dose of 1 mg/kg s.c. (Rollema et al., 2009) will be an order of magnitude higher than steady state human plasma levels after 1 mg BID (Faessel et al., 2006), while trough levels at night will be below therapeutic human plasma levels. Another limitation is that the radioligand IBZM binds with equal affinity to DRD2 and DRD3 (Videbaek et al., 2000). The ventral striatum expresses more DRD3 compared to DRD2 than the dorsal striatum, which is true both for rat and human brain (Booze and Wallace, 1995; Murray et al., 1994). Since we presently show different temporal and dosing effects of varenicline for the dorsal and more ventral parts of the striatum, it is tempting to speculate that varenicline may induce different effects on the expression of DRD2 vs DRD3. However, further studies using selective tracers for DRD2 and DRD3 are necessary to test this hypothesis.

Finally, we would like to note that imaging studies in drug dependent subjects have shown a decrease of about 15% in striatal DRD2/3 receptor binding in cocaine-dependent patients compared to controls (Martinez et al., 2004, 2009). As varenicline resulted in a higher DRD2/3 receptor availability by about 13% in dorsal and 11% in nucleus accumbens in rats compared to control rats, and it may have the potential to normalize decreased DRD2/3 availability in drug or nicotine dependence. Therefore, we propose future studies in drug-addicted rats or mice addressing the potential of varenicline to normalize DRD2/3 availability in drug-addicted study populations. Moreover, the present study was performed in genetically identical rats. The study by Noble et al. (1991) showed decreased DRD2 receptors in brains of alcoholics only when carrying the DRD2 A1 allele. Therefore, further human studies on the mechanisms of action of varenicline should take into account potential effects of genes for DRD2/3 receptors.

In conclusion, administration of varenicline resulted in a significantly higher striatal DRD2/3 availability in rats compared to control rats and these effects are long-lasting. Our findings support the potential of partial nAChR agonists, such as varenicline, in the treatment of populations with low DRD2/3 availability, including drug-dependent patients other than smokers, possibly extending to other reward deficiency syndromes like obesity and other impulsive and compulsive behaviours.