Development of new neurobiological strategies to treat patients with cocaine dependence
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Chapter 3

Varenicline increases striatal dopamine $D_{2/3}$ receptor binding in rats

From:
Increasing dopamine D\(_{2/3}\) receptor availability is postulated to be a treatment for drug addiction. Varenicline, an \(\alpha 4\beta 2\)-nicotinic partial agonist, is effective for nicotine dependence. We hypothesize that varenicline increases dopamine D\(_{2/3}\) receptor availability. Twenty male drug-naïve rats were randomized to varenicline (2 mg/kg) or placebo for 14 days, and then injected with the dopamine D\(_{2/3}\) radiotracer \[^{123}\text{I}\]IBZM. We found significantly higher striatum-to-cerebellum binding ratios in both dorsal and ventral striatum for the varenicline group compared with placebo. Varenicline increases dopamine D\(_{2/3}\) receptor availability in drug-naïve rats. Therefore, varenicline may be an effective treatment for addictions other than smoking.
**Introduction**

Varenicline (7,8,9,10-tetrahydro-6,10-methano-6Hpyrazino-(2,3-h)(3)-benzazepine) is a high-affinity $\alpha_{4}\beta_{2}$ nicotinic acetylcholine receptor partial agonist registered and effective for the treatment of nicotine dependence (Eisenberg et al. 2008). Low dopamine (DA) $D_{2/3}$ receptor availability increases the risk for developing drug addiction in non-human primates (Nader et al. 2006). High DA $D_{2/3}$ availability correlates with unpleasant effects of stimulants in humans (Volkow et al. 1999) and decreases cocaine and alcohol self-administration in rats (Thanos et al. 2005, 2008). Finally, $\alpha_{4}$-nicotinic receptor knockout mice show higher DA $D_2$ receptor availability compared with wild types, although not significant (Parish et al. 2005). We, therefore, hypothesize that DA $D_{2/3}$ receptor availability can be increased by modulating nicotine receptors using varenicline. This is important because pharmacologically increasing DA $D_{2/3}$ receptor availability in brain areas such as the striatum might be an effective approach for treating drug addiction (Thanos et al. 2005, 2008; Nader et al. 2006). In the current study, we examine the effect of varenicline on DA $D_{2/3}$ receptor availability in the drug-naive rat striatum using $[^{123}\text{I}]$IBZM ex-vivo storage phosphor imaging. We hypothesize that sustained administration of the nicotinic receptor partial agonist varenicline leads to up-regulation of striatal $D_{2/3}$ receptors.

**Materials and methods**

Male Wistar rats were randomized to either s.c. injections of saline ($n = 10$) or 2 mg/kg varenicline ($n = 10$) for 14 consecutive days at approximately the same time each morning. One day later, rats were anesthetized and administered 0.3 ml ± 0.1 ml $[^{123}\text{I}]$IBZM (37.7 MBq ± 2.8 MBq) i.v. through the tail. Ninety minutes later (Verhoeff et al. 1991), rats were sacrificed by bleeding through heart puncture under anesthesia. Brains were removed and sliced into 50 mm slices. Storage phosphor imaging was performed as described earlier (Knol et al. 2008). We exposed the plates with tissue sections for an average of 16.7 ± 1.2 hours. Regions of interest were drawn accordingly to standard rat brain atlas (Paxinos & Watson 1986) and analyzed using AIDA image analysis (Fig. 1). We
selected eight consecutive slices with highest binding (expressed as photostimulated luminescence) for both left and right dorsal striatum of each rat. For the ventral striatum, two consecutive slices were selected. For the cerebellum, seven consecutive slices with highest binding were selected as area of non-specific binding (Verhoeff et al. 1993). Ratios of striatum-to-cerebellum binding were obtained by using the averaged uptake of left and right parts and were calculated as follows: \[
\frac{\text{(left + right) striatum}}{2} / \text{cerebellum}.
\]

Varenicline (1 mg film-coated tablets, Pfizer Limited, Kent, UK) was dissolved in ethanol and extended with 0.9% natriumchloride solution. The obtained 0.1 ml/100 g weight solution was refrigerated during the experiment. \[^{123}\text{I}]\text{IBZM} \text{ (GE Healthcare, Eindhoven, the Netherlands) had a specific activity of 550 MBq/mol and a radiochemical purity of > 95%. Anesthesia contained a 2 : 1 ketamine/xylazine mix.}

Outcomes were analyzed using SPSS 15 (SPSS Inc, Chicago, IL, USA). Data were normally distributed and t-tests were used to assess within- and between-group differences with a probability value of 0.05. All procedures were approved by the Animal Ethics Committee (AMC, Amsterdam, the Netherlands).

**Figure 1:** Storage phosphor image of a control rat. Binding of \[^{123}\text{I}]\text{IBZM} \text{ (expressed as photostimulated luminescence) to dopamine D}_2/D_3 \text{ receptors in the dorsal striatum (a) and regions of interest positions of right and left dorsal striatum and cerebellum (b).}
Results

One animal was excluded from analysis because of premature death during $[^{123}\text{I}]$IBZM injection. For the dorsal striatum, we found mean specific ratios of $4.8 \pm 0.5$ (average ± standard deviation) for the placebo group, and binding ratios of $5.5 \pm 0.5$ for the varenicline group. This corresponds to a relative increase of $13.9\%$ in $D_{2/3}$ binding ratios significant at $P = 0.014$ (95% CI: 3.2%; 24.6%; Fig. 2). For the nucleus accumbens (ventral striatum), we found specific striatum-to-cerebellum ratios of $3.0 \pm 0.4$ for the placebo-treated group and $3.4 \pm 0.2$ for the varenicline-treated group. This represents a relative increase of $14.7\%$ in varenicline-compared with placebo-treated rats at $P = 0.009$ (95% CI: 4.1%; 25.3%; Fig. 2).

Figure 2: Dorsal and ventral mean striatum to cerebellum $[^{123}\text{I}]$IBZM binding ratios for saline- and varenicline-treated rats. All data represent means ± SD, *$p<0.05$. 

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Conclusions

Rats treated with varenicline daily for 14 consecutive days with 2 mg/kg had significantly increased levels of DA D2/3 receptor availability in both dorsal (13.9%) and ventral striatum (14.7%). Several studies point out that addiction treatment studies should focus on enhancing D2 availability (Thanos et al. 2005; Nader et al. 2006; Thanos et al. 2008).

Discussion

The current experiment shows the ability of a nicotinic acetylcholine receptor partial agonist to increase striatal DA D2-like receptor density. This supports the role of non-dopaminergic drugs in enhancing DA receptor availability in the striatum by manipulating α4β2 nicotinic acetylcholine receptors using the partial agonist varenicline. Here, rats were not previously exposed to addictive drugs. Consequently, it is likely to find effects of varenicline on DA D2 receptor availability larger than 15% in rats previously exposed to drugs.

There were several limitations to the current study; future studies should use several dosages, variable treatment duration and variable post-treatment interval assessments. This would enable assessment of dose dependency, effect of treatment duration and longevity of increased DA receptor availability after varenicline administration. In the current study, rats were anesthetized prior to [123I]IBZM injection. Possible changes induced by the mix of anaesthetics would, however, have occurred in both varenicline- and saline-treated groups, and would not have affected varenicline-treated rats differently compared with saline-treated rats. Furthermore, it might be more cogent to use base material rather than crushing the tablets. Our method, however, is a good representation of the human situation. In conclusion, in the current experiment, 2 mg/kg s.c. varenicline injections for 14 consecutive days produces a significant increase in D2/3 availability. Therefore, varenicline is not only an effective drug for the treatment of nicotine dependence, but it may also become an effective treatment for other drug addictions.