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# Effect of Chronic Nicotine Treatment and Withdrawal on Rat Striatal $D_1$ and $D_2$ Dopamine Receptors

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**Abstract**—The effects on rat striatal dopamine receptors after chronic nicotine administration (3 and 12 mg $kg^{-1}$ $day^{-1}$), and after withdrawal from chronic nicotine (12 mg $kg^{-1}$ $day^{-1}$), were studied. After 21 days of continuous minipump infusion, the control (saline) and nicotine-treated rats were killed. The nicotine-withdrawal rats were killed on day 28, 7 days after pump removal. Radioligand studies were performed to determine $D_1$ ([$^3$H]SCH23390) and $D_2$ ([$^3$H]spiperone) striatal dopamine receptor affinity ($K_d$) and maximum binding ($B_{max}$). Dopamine inhibition of antagonist binding at 3 concentrations and the effect of 0·3 mM GTP on binding affinity were examined. No statistically significant differences between control and nicotine treatment or withdrawal groups were noted in either $D_1$ or $D_2$ receptor $K_d$ or $B_{max}$. Although nicotine has been shown to affect nigrostriatal dopamine release, chronic treatment does not appear to alter overall striatal dopaminergic receptor binding parameters.

Despite the well-known risks of smoking, approximately one third of the adult population of the USA continues to smoke tobacco (Pomerleau & Pomerleau 1984). Nicotine, considered to be the primary psychoactive ingredient in tobacco smoke (Clarke 1987), rapidly enters the central nervous system (Romano et al 1981). Multiple neurotransmitter systems are affected by nicotine (Balfour 1982), and nicotinic receptors have been noted to be present in high densities in multiple brain regions including the caudate (Adem et al 1989).

Recent clinical observations have noted that there is an apparent negative association between smoking and Parkinson's disease (Baron 1986), while a positive association appears to exist between smoking and tardive dyskinesia (Binder et al 1987; Yassa et al 1987; Kirch et al 1988). Given that abnormalities in nigrostriatal dopaminergic function are thought to be central to both disorders, the observed correlations with smoking make it important to examine more carefully the effects of nicotine on this neurotransmitter system.

Studies that have examined the effects of acute administration of nicotine in-vivo and in-vitro suggest that dopamine release may be facilitated by nicotine (Westfall 1974; Sakurai et al 1982). Chronic treatment studies, however, indicate that dopamine turnover may decrease with longer exposure to nicotine (Kirch et al 1987; Fung & Lau 1989).

Preliminary reports on the effects of chronic nicotine treatment on striatal dopamine receptors have produced varied results, with some noting no effect (Reilly et al 1987), others reporting apparent upregulation of receptors (Fung & Lau 1988, 1989; Wiener et al 1989), and one report suggesting that nicotine prevented haloperidol-induced receptor supersensitivity (Prasad et al 1989). In an effort to define better the effects of chronic nicotine on both $D_1$ and $D_2$ dopamine receptors in an animal model of chronic drug administration and withdrawal, we treated rats with nicotine at two doses via constant infusion for 3 weeks (with an additional group of animals treated for 3 weeks then withdrawn from nicotine for 1 week), and then assessed striatal dopamine receptor binding.

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## Materials and Methods

*Chronic nicotine administration*

After 1 week of acclimatization, 30 male Sprague-Dawley rats, 290–340 g, were divided into 4 groups, with 7 rats receiving a control 0·9% NaCl (saline) solution, 8 receiving nicotine 3 mg $kg^{-1}$ $day^{-1}$, and 15 receiving nicotine 12 mg $kg^{-1}$ $day^{-1}$. All rats were killed after the 21st day of minipump infusion except 7 animals in the 12 mg $kg^{-1}$ $day^{-1}$ group. These 7 rats had their nicotine minipumps removed after 21 days and then were killed 7 days later. Rats were housed in wire cages with food and water freely available. Smoking in the animal housing area was not allowed.

Solutions of nicotine (free base, Sigma Chemical, St Louis, MO, USA) prepared for delivery at the above doses were diluted in sterile saline and loaded into osmotic minipumps (Alzet model 2M44; Alza Corp., Palo Alto, CA, USA) designed to deliver the drug at a constant rate for 28 days. The pumps were implanted s.c. behind the shoulder (and were removed from the drug withdrawal group) under methoxyflurane anaesthesia. The self-contained pumps allowed unrestricted activity with no handling of animals except for pump implantation and removal.

After 21 days of infusion (28 days for the nicotine withdrawal group) each animal was decapitated and the brain quickly removed and dissected on ice. The hemispheres were separated and both the right and left striatum were removed entirely. The striata from each animal were transferred to a preweighed conical tube and immediately frozen. Samples were stored at −60°C until assayed.

*Radioreceptor binding assays*

Individual rat striata from treatment or control animals were homogenized in 50 volumes (w/v) of ice-cold 50 mM Tris HCl

(pH 7·7 at 25°C) using a Tekmar Tissumizer (setting 7, 10 s), washed twice by centrifugation (30 000 *g*, 4°C, 20 min) and resuspended in fresh assay buffer. For $D_1$ or $D_2$ dopamine receptor subtype saturation and competition analyses, incubations were initiated by adding rat striata (0·15–0·2 mg protein/tube) to duplicate tubes containing 4 different concentrations of [$^3$H]SCH23390 (0·1–2·0 nM) or 0·025 to 0·2 nM [$^3$H]spiperone to yield a 2·5 mL final assay volume in an assay buffer of 50 mM Tris HCl, 5 mM $MgSO_4$, 0·5 mM ethylenediaminetetraacetic acid (EDTA), and 0·02% ascorbic acid (pH 7·4 at 37°C); nonspecific binding was defined by 1 μM *cis*-flupentixol. [$^3$H]SCH23390 (72·5 Ci mmol$^{-1}$) and [$^3$H]spiperone (72·9 Ci mmol$^{-1}$) were purchased from Du Pont, NEN Research Products (Boston, MA, USA). All [$^3$H]spiperone binding analyses were conducted in the presence of 40 nM ketanserin to preclude the binding of [$^3$H]spiperone to 5-$HT_2$ receptors. For $D_1$ and $D_2$ dopamine competition assays, 0·25 nM [$^3$H]SCH23390, 0·1 nM [$^3$H]spiperone, 3 concentrations of dopamine (0·1, 1·9 and 10 μM for the $D_1$ assay, and 0·01, 0·1 and 1·0 μM for the $D_2$ assay), and 0·3 mM GTP were used. All test tubes were incubated at 37°C for 30 min and filtered under vacuum through Whatman GF/C glass-fibre filters that were then washed rapidly three times with 5 mL of cold 50 mM Tris HCl buffer using a modified Brandel cell harvester. The filters were collected, using a Brandel dispenser, in plastic mini-scintillation vials with 5 mL of Ecolume (ICN Biomedical, Inc, USA). Radioactivity trapped on the filters was counted using a Beckman LS 7500 liquid scintillation spectrometer at an efficiency of 42%. The amount of protein was measured according to Lowry et al (1951). $B_{max}$ and $K_d$ values for the binding of the [$^3$H]ligands to the two dopamine receptor subtypes were determined by Scatchard analyses (Scatchard 1949) of saturation data. Although tissue limitations restricted the number of data points for these studies, the correlation coefficients for the Scatchard plots were excellent ($r^2 = 0{\cdot}969 \pm 0{\cdot}08$, n = 12) for binding to a single site for each receptor. Between group comparisons were performed by Student's *t*-test.

## Results

The mean $K_d$ and $B_{max}$ for specific [$^3$H]SCH23390 and [$^3$H]spiperone binding in the 4 groups are recorded in Table 1. There were no significant differences between controls and either nicotine treatment or nicotine withdrawal animals in striatal $D_1$- and $D_2$-receptor density or affinity. There were no statistically significant differences in dopamine's inhibition of radioligand binding at any concentration of dopamine, and the presence of 0·3 mM GTP decreased the inhibition of binding of the antagonists [$^3$H]SCH23390 (Table 2) and [$^3$H]spiperone (Table 3) by $10^{-5}$ M dopamine or $10^{-6}$ M dopamine, respectively, in a similar fashion in all groups studied.

## Discussion

These results indicate that neither nicotine treatment nor withdrawal altered the absolute numbers of either $D_1$ or $D_2$ dopamine receptors in rat striatum (Tables 1–3). Neither was the affinity of dopamine for its receptors affected nor was the ability of guanosine triphosphate to reduce dopamine's affinity altered; these are parameters thought to be determined by the association of the receptors' recognition sites with their respective G-proteins. The apparent absence of changes in dopamine receptors would suggest that any direct interaction of nicotine with dopaminergic systems is more likely to involve presynaptic mechanisms or modulation of the second messenger cascades in the postsynaptic neurons.

While the results of the present study indicate that there is no significant effect of nicotine on rat striatal $D_1$- or $D_2$-

Table 1. Mean $K_d$ (nM) and $B_{max}$ (fmol (mg protein)$^{-1}$) (± s.e.m.) of [$^3$H]SCH23390 and [$^3$H]spiperone binding to $D_1$ and $D_2$ dopamine receptors, respectively, in rat striata in control animals (n = 7), animals treated with nicotine 3 mg kg$^{-1}$ day$^{-1}$ for 21 days (n = 8), animals treated with nicotine 12 mg kg$^{-1}$ day$^{-1}$ for 21 days (n = 8), and animals treated with nicotine 12 mg kg$^{-1}$ day$^{-1}$ for 21 days then withdrawn for 7 days (n = 7). No significant differences were observed between groups.

| | | Nicotine treatment (mg kg$^{-1}$ day$^{-1}$) | | |
|---|---|---|---|---|
| | Control | 3 | 12 | 12 + withdrawal |
| $D_1$-Receptor [$^3$H]SCH23390 | | | | |
| $K_d$ | 0·42 ± 0·03 | 0·39 ± 0·03 | 0·45 ± 0·05 | 0·42 ± 0·04 |
| $B_{max}$ | 1229 ± 84 | 1085 ± 37 | 1165 ± 78 | 1017 ± 26 |
| $D_2$-Receptor [$^3$H]Spiperone | | | | |
| $K_d$ | 0·046 ± 0·005 | 0·047 ± 0·008 | 0·051 ± 0·005 | 0·045 ± 0·007 |
| $B_{max}$ | 248·7 ± 15·1 | 224·1 ± 16·8 | 248·4 ± 18·8 | 208·5 ± 15·0 |

Table 2. $D_1$-Receptor binding (% of control) in the presence of dopamine (M). No significant differences were observed between groups.

| | Dopamine (M) | | | |
|---|---|---|---|---|
| Nicotine treatment (mg kg$^{-1}$ day$^{-1}$) | $10^{-7}$ | $10^{-6}$ | $10^{-5}$ | $10^{-5}$ + 0·3 mM GTP |
| Control | 74·8 ± 3·4 | 42·6 ± 2·1 | 13·9 ± 0·8 | 30·8 ± 0·5 |
| 3 | 72·9 ± 1·8 | 42·6 ± 1·6 | 12·8 ± 0·8 | 31·5 ± 0·6 |
| 12 | 69·1 ± 2·3 | 41·3 ± 0·7 | 12·9 ± 0·6 | 30·7 ± 0·9 |
| 12 + 7 day withdrawal | 72·1 ± 1·1 | 40·0 ± 0·1 | 11·6 ± 0·4 | 28·2 ± 0·4 |

Table 3. $D_2$-Receptor binding (% of control) in the presence of dopamine (M). No significant differences were observed between groups.

| Nicotine treatment (mg kg$^{-1}$ day$^{-1}$) | Dopamine (M) $10^{-8}$ | $10^{-7}$ | $10^{-6}$ | $10^{-6}$ + 0·3 mM GTP |
|---|---|---|---|---|
| Control | 70·0 ± 5·8 | 31·9 ± 7·3 | 10·6 ± 2·9 | 45·4 ± 3·2 |
| 3 | 63·5 ± 2·9 | 26·1 ± 2·5 | 9·3 ± 1·9 | 43·4 ± 2·3 |
| 12 | 68·6 ± 4·7 | 25·1 ± 3·0 | 8·9 ± 1·6 | 41·4 ± 2·3 |
| 12 + 7 day withdrawal | 62·9 ± 2·4 | 24·0 ± 2·3 | 7·9 ± 1·6 | 38·8 ± 1·7 |

receptors, other studies examining the effect of nicotine on dopamine receptors have produced different findings. In a series of experiments using implanted infusion minipumps prepared to deliver nicotine at 1·5 mg kg$^{-1}$ day$^{-1}$, Fung & Lau (1986, 1988, 1989) observed significant increases in $D_2$-receptor $B_{max}$ in rats treated for 14 (but not for 1 and 5) days, and observed no changes in $K_d$. $D_1$-Receptors were not examined in their studies. They did report a decrease in dopamine turnover at 14 days, results which are in agreement with our own earlier observation of decreased striatal turnover (Kirch et al 1987). In an attempt to examine the combined effects of nicotine treatment and MPTP lesions on dopamine receptors in mice, Wiener et al (1989) noted a 27% increase in the $D_2$ binding activity, with no changes in $D_1$-receptors as a result of twice-daily injections of nicotine 0·4 mg kg$^{-1}$ for 7 to 9 days. They observed no change in the $D_1$/$D_2$ ratio in spite of the reported increase in $D_2$ binding. Other investigators administered nicotine via drinking water to deliver a mean of 0·7 mg kg$^{-1}$ day$^{-1}$ for 6 weeks (Prasad et al 1989). When haloperidol was co-administered in these experiments, the nicotine treatment appeared to block the increase in $D_2$-receptor $B_{max}$ observed with haloperidol treatment alone. However, the effect of nicotine treatment alone was not examined, and the animals treated with haloperidol had been injected daily and thus subjected to additional stress and handling. Reilly et al (1987) administered nicotine to rats for 6 weeks by injection (0·8 mg kg$^{-1}$ day$^{-1}$, 5 days/week) and noted no change in the $K_d$ or $B_{max}$ of domperidone binding.

Most of the previous studies reported used relatively low doses of nicotine compared with the doses of 3 to 12 mg kg$^{-1}$ day$^{-1}$ used in the present study and shown to have a significant effect on striatal dopamine release (Kirch et al 1987). In addition, length of treatment in these studies varied from 1 day to 6 weeks, and some relied on injection (either of nicotine itself or coadministered haloperidol), a potential source of stress to the animals which may itself alter dopaminergic turnover (Urakami et al 1988). The design of the present study used minipump infusion to maintain relatively constant nicotine concentrations and to avoid the stress of handling and injections for a full 3 weeks of treatment. We also included a drug-withdrawal group to assess changes in sensitivity related to discontinuation of treatment.

The question of how nicotine and the dopamine system interact is not trivial. A leading hypothesis of the pathogenesis of tardive dyskinesia is based on neuroleptic-induced striatal dopamine receptor supersensitivity (Gerlach & Casey 1988; Smith 1988), but other central nervous system transmitters, especially noradrenaline and $\gamma$-aminobutyric acid, have been implicated in some alternative pathophysiologic hypotheses of tardive dyskinesia (Thaker et al 1987; Gerlach & Casey 1988; Smith 1988). Nicotine is a drug which is known to affect multiple central neurotransmitter systems in addition to dopamine, including a number of peptides (Balfour 1982), and regional striatal differences in dopaminergic function may be relevant to disorders such as Parkinson's disease and tardive dyskinesia (Joyce et al 1986; Hassan & Thakar 1988). Our own study and the others cited here regarding the effects of nicotine on dopamine receptors have examined whole striata rather than specific regions. Despite these limitations, however, the observed associations between smoking and neuropsychiatric movement disorders make it important that further basic and clinical studies be carried out to resolve the question of whether central nervous system effects of nicotine on dopamine or other neurotransmitters may directly affect Parkinson's disease and tardive dyskinesia.

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