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# β2* and β4* nicotinic acetylcholine receptor expression changes with progressive parkinsonism in non-human primates

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**Autoradiography was used to investigate nicotinic acetylcholine receptor (nAChR) binding in the brains of two groups of macaque monkeys with parkinsonism produced by different types of MPTP exposure: animals with cognitive deficits but no motor symptoms (motor-asymptomatic) and animals with typical motor symptoms of parkinsonism (motor-symptomatic). Motor-asymptomatic animals had no significant changes in [$^{125}$I]epibatidine binding to β2*–β4* nAChRs and [$^{125}$I]A85380 binding to β2* nAChRs in cognition-related cortical regions such as Broadman's area 46, orbitofrontal cortex, the anterior cingulate sulcus and the hippocampus, but binding of both radioligands was decreased 70–80% in the caudate and putamen. Motor-symptomatic animals had decreases in β2* and β4* nAChR in the principal sulcus (40–60%), anterior cingulate sulcus (30–55%), and orbitofrontal cortex (30–41%), but not in the hippocampus, plus significant decreases in binding (70–80%) in the caudate and putamen. These results suggest that while nAChR expression is similarly decreased in the striatum of motor-asymptomatic and motor-symptomatic MPTP-treated monkeys, there are differences in β2* and β4* nAChR expression in cortical regions in these two conditions. Therefore, our data suggest that a therapeutic strategy based on nAChR agonist administration that might improve cognition in early PD patients may, due to a changing nAChR profile, have little or no effect on the same symptoms in more advanced patients.**


*Keywords:* *Macaca fascicularis*; *Macaca mulatta*; *Macaca nemestrina*; Epibatidine; A85380

## Introduction

Neuronal nicotinic acetylcholine receptor (nAChR) agonists have been investigated for treating neurological disorders such as Parkinson's disease (PD), schizophrenia, Tourette's syndrome, mild cognitive impairment and Alzheimer's disease (Buccafusco, 2004; Lloyd and Williams, 2000). Unfortunately, the efficacy of nicotinic acetylcholine receptor (nAChR) agonists on motor and cognitive deficits in PD patients and in animals models has been mixed (Fagerstrom et al., 1994; Moll, 1926; Rusted et al., 2000; Schneider et al., 1998a,b, 1999b; Schneider et al., 2003). In order to support further development of specific drugs to complement current PD therapeutics, changes in specific nAChR populations after different degrees of nigrostriatal degeneration need to be further characterized.

Parkinson's disease is a progressive neurological disorder resulting from dopaminergic degeneration. In addition to motor symptoms that are the signature of PD, many patients also exhibit cognitive deficits unrelated to dementia or severe depression (Lees and Smith, 1983; Levin et al., 1989; Owen et al., 1992). These deficits resemble those seen with frontal lobe dysfunction, and include problems in attention set shifting, executive functions, short-term recall, spatial memory, and distractibility (Cooper et al., 1991; Flowers and Robertson, 1985; Freedman and Oscar-Berman, 1986; Pillon et al., 1989; Sharpe, 1990, 1992). Many of these cognitive deficits can be reproduced in a monkey model of early parkinsonism (Schneider et al., 1999b). Although a variety of drugs are available to alleviate the motor symptoms of PD, currently there are few options to effectively treat cognitive dysfunction in these patients (Forgacs and Bodis-Wollner, 2004).

We have previously shown that chronic low doses of the dopaminergic neurotoxin 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes monkeys to develop stable cognitive deficits analogous to those experienced by PD patients (Schneider and Kovelowski, 1990). We have also shown that the β4*-selective nAChR agonist SIB-1553A improved both attentional and memory deficits in these animals (Schneider et al., 2003), whereas the β2*-selective nAChR agonist SIB-1508Y only improved attentional deficits (Schneider et al., 1998a,b, 1999b). SIB-1508Y did not have an effect upon a measure of executive functioning in animals that also had significant parkinsonian motor symptoms (Schneider et al., 1998a,b).

In this report, we used two radioligands to investigate β2* and β4* nAChR expression in normal animals and two groups of monkeys given MPTP. [$^{125}$I]Epibatidine binds to nAChRs contain-

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ing combinations of α2–α6 and β2–β4 subunits (Alkondon and Albuquerque, 1995; Davila-Garcia et al., 1997; Houghtling et al., 1995). [$^{125}$I]A85380 binds to β2*-containing receptors(Kulak et al., 2002c; Musachio et al., 1998; Sullivan et al., 1996). Differences in quantitative autoradiography using these two radioligands can be used to elucidate changes in β2* and β4* nAChR expression. nAChRs containing these subunits have a high affinity for nicotine, are involved in the reinforcing properties of nicotine (β2), and may provide a therapeutic target for treatment and or neuroprotection in PD (Buccafusco, 2004). Significant decreases in β2*–β4* containing nAChRs have been reported in the striatum of parkinsonian squirrel monkeys administered large doses of MPTP over a relatively short period of time (Kulak et al., 2002a). In contrast, the two models of PD that were used in the current report represent an early, pre-motor-symptomatic form of the disorder and a slowly progressing, motor-symptomatic form typical of mid-stage PD patients (Schneider, 1990). Our work focused on brain regions believed to be primarily associated with the cognitive and motor deficits of PD: the hippocampus, caudate and prefrontal cortex for cognitive deficits and the motor-related cortices (premotor and motor cortex) and putamen for motor deficits (Schneider et al., 1998b, 1999b).

## Materials and methods

### *Materials*

[$^{125}$I]A85380 (5-[$^{125}$I]Iodo-3(2(*S*)-azetidinylmethoxy) pyridine, 1200–2200 Ci/mmol) was prepared as described (Kulak et al., 2002c; Musachio et al., 1998) and [$^{125}$I]epibatidine (2200 Ci/mmol) purchased from PerkinElmer, Boston, MA. Nicotine hydrogen tartrate and MPTP were obtained from Sigma, St. Louis, MO.

### *Animals*

Brain tissue from twenty adult macaque monkeys (*Macaca fascicularis*, *Macaca mulatta*, and *Macaca nemestrina*) were used for this study. Monkeys were individually housed and maintained in compliance with NIH, USDA and IACUC guidelines for the care and use of nonhuman primates. The majority of the animals were wild caught; age was estimated to be young adult based upon size, weight and dentition. Nine normal control animals were used (7 *M. fascicularis*, 1 *M. mulatta*, and 1 *M. nemestrina*). No significant autoradiographic differences were observed between species and data were subsequently pooled for analysis. All MPTP-treated animals were *M. fascicularis*; we used tissue from 4 motor-asymptomatic and 7 motor-symptomatic animals. Although some of the monkeys were previously used in behavioral research projects (Schneider et al., 1998b), all were drug-free for at least 6 months prior to being euthanized. Our MPTP administration protocols have been described in detail elsewhere (Kulak and Schneider, 2004), and are summarized in Table 1. All animals were euthanized by sodium pentobarbital overdose (150 mg/kg, i.v.). Brains were rapidly removed and bisected along the midline, one hemisphere was fixed in ice-cold 4% paraformaldehyde in 0.1 M sodium phosphate buffer for immunohistochemistry and the other hemisphere was snap frozen in isopentane on dry ice and stored at −80 °C until use.

### *[$^{125}$I]A-85380 and [$^{125}$I]epibatidine autoradiography*

Twenty μm thick brain sections were prepared at −20 °C using a Microm cryostat. They were thaw mounted onto Superfrost Plus slides (Fisher, Pittsburgh, PA), dried, and stored at −80 °C. Binding experiments were carried out as previously described (Kulak et al., 2002c). Brain sections were thawed and incubated at room temperature for 60 min in buffer (50 mM Tris, pH 7.0, 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$) plus 0.1 nM [$^{125}$I]A85380.

Nonspecific binding was performed for each rostral to caudal level and each treatment condition and was defined in the presence of 10 μM nicotine. It was indistinguishable from unexposed areas of the film. Following incubation, the sections were washed 2 × 5 min in buffer at 4 °C and 1 × 10 s in ice cold deionized $H_2O$. After drying at room temperature, slides were exposed for 1–2 days to Kodak MR film (PerkinElmer Life Sciences, Shelton, CT), simultaneously with known [$^{125}$I]standards (GE Healthcare, Piscataway, NJ).

[$^{125}$I]Epibatidine binding was done as previously described (Kulak et al., 2002a), and conditions were identical to those for [$^{125}$I]A85380 with the following exception: sections were incubated for 40 min at room temperature with 0.03 nM [$^{125}$I] epibatidine.

### *Data analysis and quantitation*

Macaque monkey atlases was used to identify the different brain regions studied (Paxinos et al., 2000; Szabo and Cowan, 1984). Quantitative differences in radioligand binding were determined by computer-assisted densitometry (BRAIN, version 4.0; Drexel University, Philadelphia, PA). Absorbance values of autoradiographic film images were corrected for background and converted to fmol/mg tissue by comparison with curves generated from known radioisotope standards exposed to film with the

Table 1
MPTP administration methods in monkeys

| Lesion type | I.V. MPTP administration | # times MPTP administration | Duration MPTP administration | Duration of motor symptoms | Parkinsonian % rating | Decrease dopamine transporter |
|---|---|---|---|---|---|---|
| Chronic low-dose MPTP, motor symptomatic | 0.05–0.15 mg/kg, then 0.3–0.5 mg/kg | 2–3× per week then 1–2× per week | 4–6 months (until cognitive deficits), then 4–7 additional months | 30–69 months | 31.2±4.4 | >95% DL, DM; >90% VL, VM |
| Chronic low-dose MPTP, motor asymptomatic | 0.01–0.15 mg/kg | 2–3× per week | 1.5–6 months none | | 1.75±0.75 | >95% DL, DM; ~59% VL, VM |

Parkinsonian motor symptom score ranges from 0 (normal) to a maximum score of 41, as described previously (Schneider et al., 1998b). Dopamine transporter integrity was measured by [$^{3}$H]mazindol binding as previously reported (Kulak and Schneider, 2004). DL, dorsolateral; DM, dorsomedial; VL, ventrolateral; VM, ventromedial.