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Loss of Nicotinic Receptors in Monkey Striatum after 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine Treatment Is Due to a Decline in α-Conotoxin MII Sites

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ABSTRACT

Nicotinic acetylcholine receptors (nAChRs) in the basal ganglia are a potential target for new therapeutics for Parkinson's disease. As an approach to detect expression of nAChRs in monkeys, we used ^{125}I-epibatidine, an agonist at nAChRs containing α2 to α6 subunits. ^{125}I-Epibatidine binding sites are expressed throughout the control monkey brain, including the basal ganglia. The α3/α6-selective antagonist α-conotoxin MII maximally inhibited 50% of binding in the caudate-putamen and had no effect on ^{125}I-epibatidine binding in the frontal cortex or thalamus. In contrast, inhibition experiments with nicotine, cytisine, and 3-(2(*S*)-azetidinylmethoxy)pyridine·2HCl (A85380) showed a complete block of ^{125}I-epibatidine binding in all regions investigated and did not discriminate between the α-conotoxin MII-sensitive and -insensitive populations in the striatum. To assess the effects of nigrostriatal damage, monkeys were rendered parkinsonian with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Animals with moderate striatal damage (dopamine transporter levels ~30% of control) had a 40 to 50% decrease in ^{125}I-epibatidine binding. Inhibition studies showed that the decrease in epibatidine binding was due to loss of α-conotoxin MII-sensitive nAChRs. Monkeys with severe nigrostriatal damage (dopamine transporter levels ≤5% of control) exhibited a 55 to 60% decrease in ^{125}I-epibatidine binding, which seemed to be due to a complete loss of α-conotoxin MII nAChRs and a partial loss of other nAChR subtypes. These results show that nAChRs expressed in the primate striatum have similar affinities for nicotine, cytisine, and A85380, that α-conotoxin MII discriminates between nAChR populations in the caudate and putamen, and that α-conotoxin MII-sensitive nAChRs are selectively decreased after MPTP-induced nigrostriatal damage.

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of dopamine neurons in the substantia nigra (Lang and Lozano, 1998). The ensuing dopaminergic deficit results in motor symptoms that are relieved with administration of the dopamine precursor L-dopa. However, motor and other complications develop with long-term use of L-dopa, and furthermore, this drug does not halt disease progression. These observations raise the need for alternative therapeutic approaches. Accumulating evidence suggests that activation of nicotinic acetylcholine receptors (nAChRs) may have therapeutic potential for PD. This is based on findings showing 1) an apparent protective effect of tobacco use on PD (Morens et al., 1995; Quik and Jeyarasasingam, 2000), 2) positive effects of nicotine administration on parkinsonian symptomatology in humans (Kelton et al., 2000) and in monkeys (Schneider et al., 1998), and 3) the ability of nicotine to stimulate dopamine release in the caudate-putamen (MacDermott et al., 1999).

Multiple nAChR subunits have been identified in the basal ganglia of rodents and nonhuman primates, including α2 to α7 and β2 to β4 (Jones et al., 1999; Quik et al., 2000a; Han et al., 2000). Although the composition of basal ganglia nAChRs is still uncertain, the presence of these transcripts would allow for numerous pentameric subunit combinations. For example, the majority of nAChRs expressed in rodent brain that bind [^{3}H]nicotine and [^{3}H]cytisine seem to be composed of α4 and β2 subunits (Flores et al., 1991; Davila-Garcia et al., 1997); however, receptor studies using [^{3}H]epibatidine, ^{125}I-α-bungarotoxin, and ^{125}I-α-conotoxin MII indicate that α7- or α3/α6-containing nAChRs are also present (Marks et al., 1986; Whiteaker et al., 2000c). In human basal ganglia, [^{3}H]nicotine, [^{3}H]cytisine, and [^{3}H]epibatidine binding sites have been identified (Gotti et al., 1997; Court et al., 2000; Perry et al., 2000), but the nAChR subtypes contributing to the binding sites have not been

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ABBREVIATIONS: PD, Parkinson's disease; nAChRs, nicotinic acetylcholine receptors; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; [^{125}I]RTI-121, 3β-(4[^{125}I]iodophenyl)tropane-2β-carboxylic acid isopropyl ester; A85380, 3-(2(*S*)-azetidinylmethoxy)pyridine·2HCl; DAT, dopamine transporter.

extensively characterized. The subtypes of nAChRs expressed in control and PD brains is an important issue when considering the therapeutic potential of nicotinic ligands. Although nAChR expression declines in the caudate-putamen and substantia nigra of PD brains (Gotti et al., 1997; Court et al., 2000; Perry et al., 2000), a large portion (30 to 70%) of the receptors remains as potential therapeutic targets.

As an approach to determine the nAChRs that may be altered with nigrostriatal degeneration in primates, we initiated a series of experiments using squirrel monkeys treated with the nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). This treatment results in loss of dopaminergic terminals in the caudate-putamen, decreased dopamine levels, and symptoms similar to idiopathic PD (Przedborski et al., 2001). For this study, we used ^{125}I-epibatidine, a ligand that binds with high affinity to nAChRs containing α2 to α6 subunits (Davila-Garcia et al., 1997). The work indicates that in MPTP-induced parkinsonism, there is a preferential loss of a specific subset of nAChRs recognized by ^{125}I-epibatidine.

Experimental Procedures

Materials. ^{125}I-Epibatidine (2200 Ci/mmol) and [^{125}I]RTI-121 (2200 Ci/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA). Nicotine hydrogen tartrate and cytisine were obtained from Sigma (St. Louis, MO), and A85380 was obtained from Fisher Scientific (Pittsburg, PA). α-Conotoxin MII was synthesized as described previously (Cartier et al., 1996).

Animals. Twenty adult, drug-free squirrel monkeys (*Saimiri sciureus*) were used for these studies (Osage Research Primates, Osage Beach, MO). They were housed individually on a 13-h light/11-h dark cycle and fed once daily with free access to water. MPTP treatment and behavioral testing was done as described previously (Quik et al., 2001). Briefly, baseline locomotor activity was evaluated daily for a 1-h period for 8 to 11 consecutive days using a computerized movement monitor cage (Quik et al., 2001). Animals were then treated with saline or 2 mg/kg MPTP s.c. Starting 2.5 weeks after treatment, locomotor activity was again measured for a 10-day period. The severity of the parkinsonian syndrome after MPTP treatment was rated using a modified monkey parkinsonian rating scale (Langston et al., 2000; Quik et al., 2000b). Five clinical parameters were evaluated including spatial hypokinesia, body bradykinesia, manual dexterity, balance, and freezing; each of which has a 5-point range with 0 being normal and 4 being severely affected, allowing for a composite score ranging between 0 (normal) to 20 (severely parkinsonian). If the parkinsonian scored was less than 3, monkeys were given a second injection of MPTP at a lower dose (1.75 mg/kg) because the animals were usually more susceptible to MPTP with the second injection. Monkeys were killed 4 weeks after the final MPTP injection in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. Ketamine hydrochloride (15–20 mg/kg i.m.) was administered to sedate the animals, followed by an injection of 0.22 ml/kg i.v. euthanasia solution (390 mg of sodium pentobarbital and 50 mg of phenytoin sodium/ml).

Tissue Preparation. The brains were removed, chilled, cut into 6-mm-thick blocks, quick frozen in isopentane on dry ice, and kept at −80°C until use. Twenty-micrometer-thick brain sections were prepared at −20°C in a Leica cryostat, thaw mounted onto poly-L-lysine coated slides, dried, and stored at −80°C. A squirrel monkey atlas (Emmers and Akert, 1963) was used to identify different brain regions in Nissl-stained tissue sections from each monkey. Level assignments indicate the distance anterior (in millimeters) to the interaural line; for example, level A15.0 is 15 mm anterior to the interaural line.

^{125}I-Epibatidine Binding. Binding was conducted as described previously (Quik et al., 2000b). Briefly, 20-μm-thick monkey tissue sections were thawed and incubated with or without competing ligand at room temperature for 40 min in buffer (50 mM Tris, pH 7.0, 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, and 1.0 mM $MgCl_2$) plus ^{125}I-epibatidine (2200 Ci/mmol; PerkinElmer Life Sciences). The concentration of radiolabeled ligand ranged from 0.01 to 0.08 nM, which is well below the K_d of 0.10 nM (P. Whiteaker, personal communication). Nonspecific binding was defined in the presence of 0.1 mM nicotine. Sections were washed twice for 5 min in buffer at 4°C and once for 10 s in ice-cold doubly distilled H_2O. After drying at room temperature, slides were exposed for 3 to 5 days to Hyperfilm β-Max film (Amersham Biosciences, Piscataway, NJ).

[^{125}I]RTI-121 Binding. Dopamine transporter (DAT) density in the caudate-putamen was assessed using [^{125}I]RTI-121 binding as described previously (Quik et al., 2001). Monkey sections were preincubated twice for 15 min in preincubation buffer (50 mM Tris-HCl, pH 7.4, 120 mM NaCl, and 5 mM KCl). Sections were then incubated

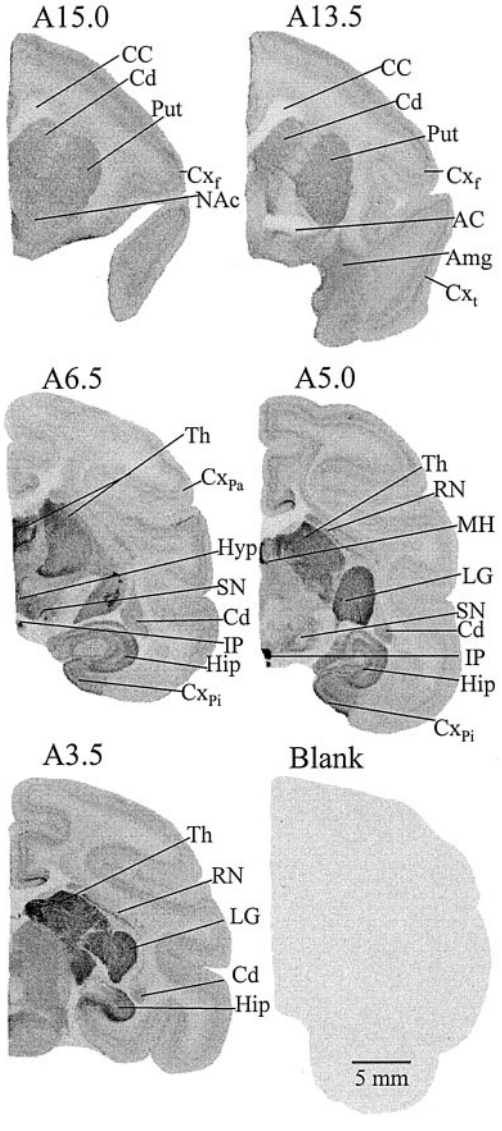

Fig. 1. Autoradiograms depicting the distribution of ^{125}I-epibatidine binding sites at different anatomical levels throughout control monkey brain (A15.0 to A3.5). Note the presence of ^{125}I-epibatidine sites in the caudate, putamen, and substantia nigra. Blank represents nonspecific binding in the presence of 100 μM nicotine. Scale bar is 5 mm. AC, anterior commissure; Amg, amygdala; CC, corpus callosum; Cd, caudate; Cx$_f$, frontal cortex; Cx$_{pa}$, parietal cortex; Cx$_{pi}$, piriformis cortex; Cx$_t$, temporal cortex; Hip, hippocampus; Hyp, hypothalamus; IP, interpeduncular nucleus; LG, lateral geniculate nucleus; MH, medial habenula; NAc, nucleus accumbens; OT, olfactory tubercle; Put, putamen; RN, reticular nucleus; SN, substantia nigra; Th, thalamus.

for 2 h with 50 pM [^{125}I]RTI-121 in preincubation buffer plus 0.025% bovine serum albumin and 1 μM fluoxetine. Nonspecific binding was determined in the presence of 100 μM nomifensine, a dopamine uptake inhibitor. Sections were washed four times for 15 min in ice-cold preincubation buffer, once for 10 s in ice-cold doubly distilled H_2O, dried, and exposed for 2 days to Hyperfilm βmax film.

Data Analysis and Quantitation. Quantitative differences in radioligand binding were determined by computer-assisted densitometry (ImageQuant, Molecular Dynamics, Sunnyvale, CA). Absorbances of autoradiographic film images were corrected for background and converted to femtomole per milligram of tissue by comparison with curves generated from known ^{125}I standards exposed to film with the sections. Absorbances for tissue sections and standards were within the linear range of the film. For the caudate and putamen, density was measured at level A14.5, A13.5, and A12.5, and values were averaged to obtain the amount of ^{125}I-epibatidine binding in each region per monkey.

K_i values were derived by the method of (Cheng and Prusoff, 1973). ^{125}I-Epibatidine inhibition curves were fit to both one- and two-site models and statistically compared to determine best fit (GraphPad Prism; GraphPad Software, San Diego, CA). All values are expressed as the mean ± S.E.M for the indicated n. For statistical analysis, one-way analysis of variance followed by Newman-Keuls multiple comparison was used with $p < 0.05$ considered significant (GraphPad Prism).

TABLE 1

Regional quantitation of ^{125}I-epibatidine binding

Coronal brain sections were prepared and binding of 0.03 nM ^{125}I-epibatidine performed, as described under *Experimental Procedures*. Each value represents the mean ± S.E.M. from four to six control monkeys.

Brain Region	Tissue	Brain Region	Tissue
	fmol/mg		*fmol/mg*
Telencephalon		Diencephalon	
Neocortex		Thalamus	
Frontal cx	1.29 ± 0.10	Laterodorsal n.	6.06 ± 0.60
Parietal cx	1.24 ± 0.08	Mediodorsal n.	6.51 ± 0.88
Temporal cx	1.06 ± 0.09	Ventrolateral n.	5.90 ± 0.38
Piriformis cx	3.30 ± 0.44	Ventroposterior n.	4.32 ± 0.26
Basal ganglia		Reticular nucleus	2.14 ± 0.11
Caudate	2.05 ± 0.02	Epi- and subthalamus	
Putamen	1.97 ± 0.04	Medial habenula	10.00 ± 0.44
Nucleus accumbens	1.83 ± 0.05	Metathalamus	
Globus pallidus	0.71 ± 0.09	Lateral gen. n.	5.64 ± 0.34
Substantia nigra	2.50 ± 0.11	Hypothalamus	3.37 ± 0.32
Hippocampus		Mesencephalon	
CA1	2.38 ± 0.20	Interpeduncular n.	13.96 ± 0.68
CA2	2.67 ± 0.20	Fiber Tracts	
CA3	2.87 ± 0.44	Ant. commissure	0.10 ± 0.01
Dentate gyrus	2.66 ± 0.30	Corpus callosum	0.07 ± 0.01
Subiculum	4.75 ± 0.27	Cerebellum	0.40 ± 0.06
Amygdala	1.52 ± 0.11		

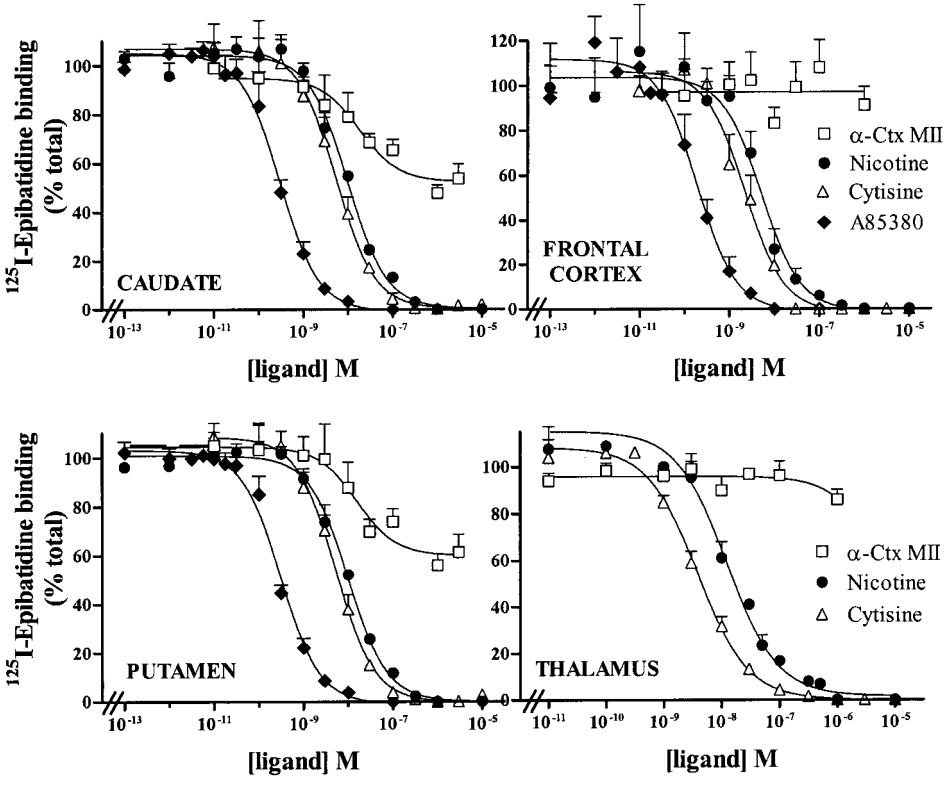

Fig. 2. ^{125}I-Epibatidine binding in control brain is differentially inhibited by nAChR ligands. Sections were incubated with ^{125}I-epibatidine in the absence or presence of 0.1 pM to 10 μM nicotine, cytisine, A85380, or α-conotoxin MII (α-Ctx MII). Note that nicotine, cytisine, and A85380 completely inhibited binding at the highest concentrations tested, whereas α-Ctx MII only blocked 50% of ^{125}I-epibatidine binding sites in the caudate-putamen and had no affect on binding in the frontal cortex or thalamus. All curves fit best to a one-site model (see Table 2 for K_i values). Points are mean ± S.E.M. of three to four experiments. If no error bars are depicted, S.E.M. was within the size of the symbol.

Results

^{125}I-Epibatidine Binding in Control Monkey Brain. The regional distribution of nAChRs was evaluated in control monkey brain using ^{125}I-epibatidine (0.03 nM). ^{125}I-Epibatidine binding sites are distributed throughout the monkey brain, with the highest density in the medial habenula, interpeduncular nucleus, and thalamus (Fig. 1; Table 1). In the basal ganglia, binding was greatest in the substantia nigra, with moderate levels in the caudate-putamen and nucleus accumbens and low levels in the globus pallidus. Nonspecific binding in the presence of 100 μM nicotine was indistinguishable from film background.

nAChR Ligands Differentially Inhibit ^{125}I-Epibatidine Binding. As an approach to identify nAChR subtypes present in monkey brain, ^{125}I-epibatidine inhibition studies were performed using nicotine, cytisine, A85380, and α-conotoxin MII (Fig. 2). Nicotine, cytisine, and A85380 completely inhibited ^{125}I-epibatidine binding in all brain regions investigated and were not different from film background at the highest concentrations tested. In contrast, α-conotoxin MII (3 μM) maximally inhibited binding by 50% in the caudate and putamen and did not affect binding in the frontal cortex or thalamus. The results suggest that monkey caudate-putamen express both α-conotoxin MII-sensitive and -insensitive nAChR populations; each comprising ~50% of the total amount of ^{125}I-epibatidine sites.

^{125}I-Epibatidine inhibition curves for nicotine, cytisine, and A85380 were fit to both one- and two-site models and statistically compared to determine best fit. In contrast to previous studies conducted in rodents, all of the drugs inhibited ^{125}I-epibatidine binding in a monophasic manner (Marks et al., 1998; Whiteaker et al., 2000b,c), suggesting that these ligands have similar affinities for the α-conotoxin MII-sensitive and -insensitive nAChR populations. K_i values for each ligand in the different brain regions are summarized in Table 2.

MPTP Treatment Resulted in Nigrostriatal Damage and Motor Deficits. The effect of MPTP treatment was evaluated biochemically by measuring DAT density in the caudate and putamen and behaviorally by monitoring baseline locomotor activity and parkinsonism (Figs. 3 and 5). MPTP-treated animals were separated into two treatment groups as follows. Monkeys with striatal dopamine transporter levels reduced by about 70% were considered moderately lesioned; they exhibited 60% declines in locomotor activity compared with pretreatment values and had only mild parkinsonism (1.57 ± 0.32). In contrast, the group designated severely lesioned had ≥95% declines in the dopamine transporter in both caudate and putamen, exhibited a ≥90% decrease in baseline locomotor activity, and were decidedly parkinsonian (7.75 ± 0.89).

Nigrostriatal Lesioning Decreases nAChR Expression in the Caudate-Putamen. Autoradiographs demonstrating the effect of MPTP treatment on nAChR and dopamine transporter expression are shown in Fig. 4. Monkeys with moderate (n = 7) and severe (n = 6) nigrostriatal damage had similar (50 and 57%, respectively) decreases in ^{125}I-epibatidine binding in the caudate and putamen (Fig. 5), whereas the frontal cortex and thalamus were unaffected (data not shown). In contrast, there were significant differences between moderate and severe treatment groups in the amount of decrease of dopamine transporter expression (70 versus 95%, $p < 0.05$).

The results described above indicate that α-conotoxin MII-sensitive nAChRs contribute ~50% toward total ^{125}I-epibatidine binding sites and that MPTP treatment decreases epibatidine binding by ~50% in both moderate and severe treatment groups. Combined with our previous work showing that moderately and severely lesioned monkeys had little or no ^{125}I-α-conotoxin MII binding, respectively (Quik et al., 2001), these results suggest that the decline in nAChR expression after nigrostriatal lesioning may be due to a selective decrease in α-conotoxin MII-sensitive nAChRs.

α-Conotoxin MII-Sensitive nAChRs Are Selectively Affected by MPTP Lesioning. We investigated the possibility that α-conotoxin MII-sensitive nAChRs are selectively decreased after nigrostriatal damage using α-conotoxin MII inhibition of ^{125}I-epibatidine binding in the

TABLE 2

Competition of ^{125}I-epibatidine binding in control and MPTP-treated monkey brain regions by nicotinic receptor ligands

MPTP treated monkeys were from the severely lesioned group. Mean ± S.E.M., n = 3 to 4 experiments.

Ligand	nAChR Specificity	Region	Control K_i	MPTP-Treated K_i
			nM	
α-Ctx MII	α3/α6	Caudate	19.3 ± 8.2	≠
		Putamen	12.2 ± 5.4	≠
		Frontal cortex	≠	≠
		Thalamus	≠	≠
Nicotine	α2–α6	Caudate	9.2 ± 0.4	4.9 ± 1.1[a]
		Putamen	9.2 ± 0.5	3.9 ± 0.6[b]
		Frontal cortex	5.3 ± 1.2	3.8 ± 1.2
		Thalamus	12.8 ± 2.1	14.5 ± 1.5
Cytisine	α4 > α2, α3, α6	Caudate	5.2 ± 1.3	2.2 ± 0.2
		Putamen	4.9 ± 1.2	2.7 ± 0.6
		Frontal cortex	4.3 ± 1.7	2.8 ± 0.9
		Thalamus	3.5 ± 0.7	3.3 ± 0.4
A85380	α4 > α2, α3, α6	Caudate	0.29 ± 0.07	0.29 ± 0.08
		Putamen	0.29 ± 0.06	0.24 ± 0.05
		Frontal cortex	0.20 ± 0.07	0.25 ± 0.05

≠, no α-conotoxin MII-sensitive component.
[a] $P < 0.01$ from control.
[b] $P < 0.001$ from control.

caudate and putamen of control, moderate, and severely lesioned monkeys (Fig. 6). The four moderate monkeys used for these experiments averaged slightly higher DAT levels (~37% of control) and ^{125}I-epibatidine binding (~75% of control) compared with the total moderate treatment group (n = 7), whereas the DAT levels (~5% of control) and ^{125}I-epibatidine binding (~40% of control) of the five severely lesioned monkeys were similar to the entire severe treatment group (n = 7). Interestingly, the inhibition curves of moderately lesioned monkeys overlap those of controls at high concentrations of α-conotoxin MII, implying that decreases in ^{125}I-epibatidine binding in these animals are due to a selective loss of α-conotoxin MII-sensitive nAChRs. α-Conotoxin MII does not inhibit ^{125}I-epibatidine binding in severely lesioned monkeys, which correlates with our previous results using ^{125}I-α-conotoxin MII (Quik et al., 2001). At 1 μM α-conotoxin MII, the amount of ^{125}I-epibatidine binding is the same for control and moderately lesioned monkeys (~50%), whereas severely lesioned monkeys show an additional ~15% decrease in ^{125}I-epibatidine binding ($p < 0.05$). This implies that α-conotoxin MII-sensitive nAChRs are selectively decreased with nigrostriatal lesioning and that severe dopaminergic deficits are necessary to affect expression of other receptor subtypes.

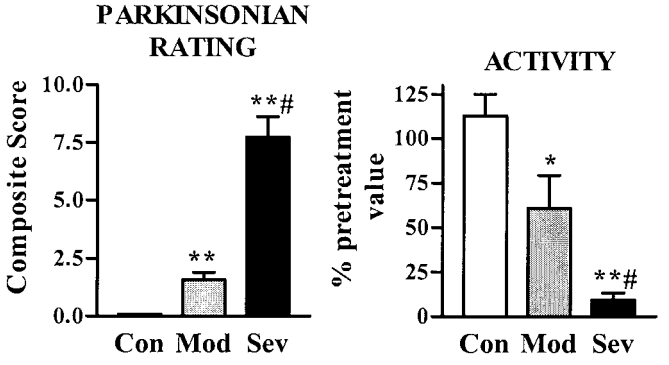

Fig. 3. Parkinson ratings and free activity in monkeys after MPTP treatment. Monkeys were administered 1.75 to 2.0 mg/kg MPTP or saline s.c., and behavioral testing was reevaluated 3 to 4 weeks after treatment, as described under *Experimental Procedures*. Monkeys were divided into control (Con), moderate (Mod), and severe (Sev) groups based upon the extent of nigrostriatal damage (see *Results*). Each value represents the mean ± S.E.M. of six to seven animals. *, $p < 0.05$ and **, $p \leq 0.01$ to control; #, $p < 0.05$ to moderates.

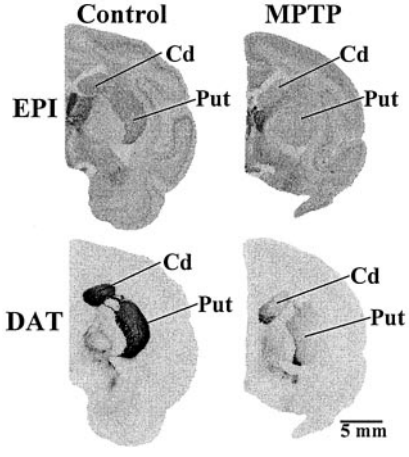

Fig. 4. MPTP treatment decreases nAChR expression in the caudate-putamen. Film autoradiograms of representative sections from a control and severely lesioned (MPTP) monkey. Note the selective decrease in ^{125}I-epibatidine binding (EPI, 0.03 nM) in the caudate (Cd) and putamen (Put). Decreases in [^{125}I]RTI-121 binding (0.05 nM) to DAT were used to evaluate the extent of nigrostriatal damage. Scale bar is 5 mm.

The ^{125}I-Epibatidine Sites That Remain in Severely Lesioned Monkeys Are Sensitive to Other nAChR Ligands. The results of nicotine, cytisine, and A85380 inhibition of ^{125}I-epibatidine binding in severely lesioned monkeys are presented in Fig. 7. As was the case with control animals, all inhibition curves in severely lesioned monkeys were monophasic. Furthermore, in all areas, the K_i values for cytisine and A85380 inhibition remained unchanged with MPTP treatment (Table 2), whereas there were statistically significant decreases in the K_i values in the caudate and putamen for nicotine ($p < 0.05$ to control, reflecting a change in the IC_{50} from 10 to 5 nM). These results provide further evidence that cytisine and A85380 do not distinguish between the α-conotoxin MII-sensitive and -insensitive nAChR populations.

Discussion

The results from the present study are the first to show that nicotinic receptors containing α6 and/or α3 are the predominant receptor population affected after moderate nigrostriatal damage in the monkey. Our data demon-

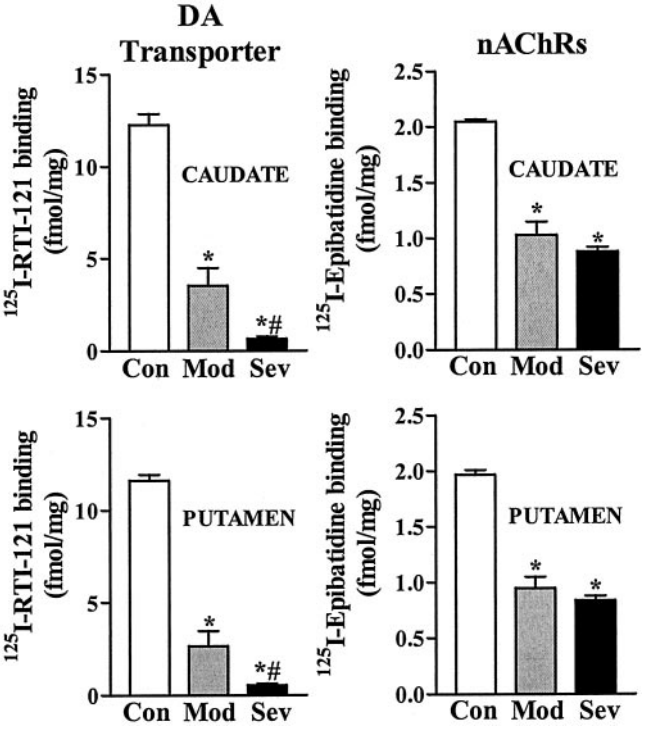

Fig. 5. Quantitative analysis of declines in nAChR and dopamine transporter expression in the caudate and putamen of parkinsonian monkeys. Monkeys were administered 1.75 to 2.0 mg/kg MPTP or saline s.c., as described under *Experimental Procedures*. Alterations in nAChR expression were investigated using ^{125}I-epibatidine binding (0.03 nM) and decreases in the dopamine (DA) transporter were studied using [^{125}I]RTI-121 (0.05 nM). Note that nAChR expression is decreased ~50% in monkeys, with either moderate (Mod, n = 7) or severe (Sev, n = 6) nigrostriatal lesions compared with controls (Con, n = 7), whereas dopamine transporter expression is differentially affected. Each bar represents the mean ± S.E.M. of the indicated number of animals. *, $p < 0.001$ compared with control; #, $p < 0.05$ to moderate.

strate that striatal ^{125}I-epibatidine sites can be subdivided into two populations of approximately equal magnitude based on sensitivity to the cone snail toxin α-conotoxin MII and that, in these latter sites, nAChRs are selectively decreased after MPTP treatment. Previous work suggested that α-conotoxin MII-sensitive receptors are present on dopaminergic neurons (Quik et al., 2001). Our present results show that in monkey striatum the nAChR populations have similar affinities for nicotine, cytisine, and A85380, that α-conotoxin MII can distinguish between nAChR populations, and that α-conotoxin MII-sensitive nAChRs comprise a large proportion (50%) of total ^{125}I-epibatidine sites. These combined data may indicate that the predominant presynaptic nAChR population on dopaminergic neurons is sensitive to α-conotoxin MII and possibly contains α3 and/or α6 subunits.

The present results, combined with our previous work (Kulak and Quik, 2000; Quik et al., 2001), suggest that at least three populations of nAChRs are expressed in monkey caudate-putamen, distinguished by their sensitivity to the nAChR ligands α-conotoxin MII and α-bungarotoxin (Table 3). α-Conotoxin MII-sensitive nAChRs contribute to 50% of ^{125}I-epibatidine binding sites, are selectively decreased with a moderate nigrostriatal lesion, and completely eliminated with severe nigrostriatal damage. α-Conotoxin MII-insensitive nAChRs also contribute to 50% of ^{125}I-epibatidine binding sites, although they are only partially decreased (20 to 25%) with severe nigrostriatal damage. The third population of nAChRs expressed in the caudate-putamen do not bind ^{125}I-epibatidine but do bind ^{125}I-α-bungarotoxin, a ligand selective for α7-containing nAChRs (Kulak and Quik, 2000). α7-Containing nAChRs do not seem to be localized to dopaminergic neurons because ≥95% dopaminergic depletion in the caudate-putamen causes ^{125}I-α-bungarotoxin binding to increase 100 to 150% (Kulak and Quik, 2000). The nAChR populations that remain in the caudate-putamen after MPTP treatment are likely to be present on nondopaminergic neurons. They may be expressed at the presynaptic terminals of glutamatergic, GABAergic, serotonergic, or other striatal afferents, or postsynaptically on striatal GABAergic or cholinergic neurons (Gotti et al., 1997; Jones et al., 1999; MacDermott et al., 1999).

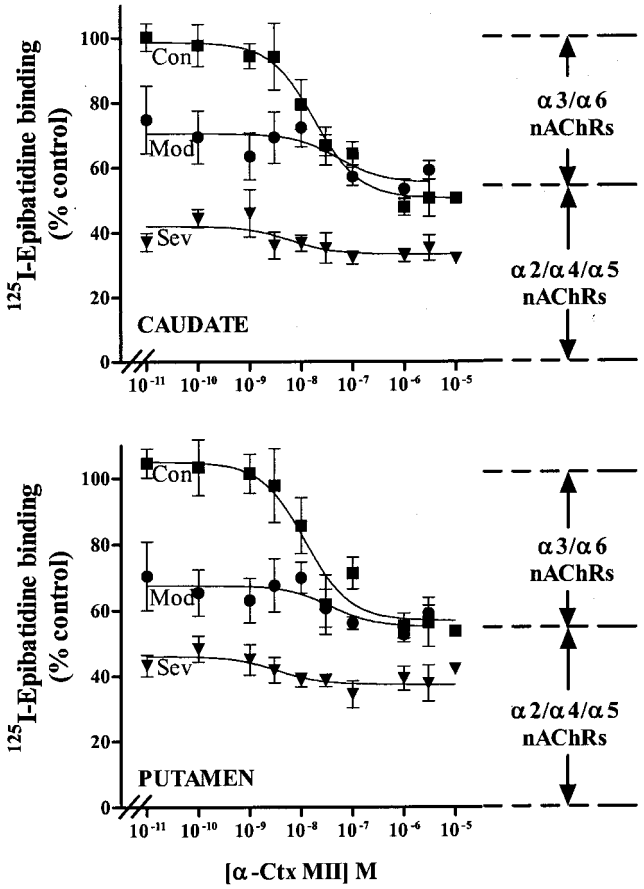

Fig. 6. α-Conotoxin MII-sensitive nAChRs are selectively decreased in the caudate-putamen after nigrostriatal damage. ^{125}I-Epibatidine inhibition studies using α-conotoxin MII were performed in the caudate and putamen of control (Con), moderate (Mod), and severely (Sev) lesioned monkeys. The putative nAChR subtypes present in the caudate-putamen, and their relative proportions in the treatment groups are indicated to the right (see *Discussion*). Points are mean ± S.E.M. of four monkeys for Mod and five monkeys for Con and Sev.

The subtypes of nAChR that bind α-conotoxin MII are currently under intense scrutiny. Early reports using *Xenopus laevis* oocytes or cell lines expressing simple α/β subunit combinations (such as α3β2 or α4β2) indicated that α-conotoxin MII preferentially binds to nAChRs with an α3β2-interface (Cartier et al., 1996; McIntosh et al., 1999). Recent work using oocytes that expressed complex nAChRs composed of multiple α and β subunits, including α6, indicates that α-conotoxin MII may also bind to nAChRs that contain an α6 subunit (Vailati et al., 1999; Kuryatov et al., 2000). Because α6 mRNA is prominently present in monkey substantia nigra (Quik et al., 2000a; Quik et al., 2000b; Han et al., 2000) and α3 mRNA is present in much lower abundance, if at all (Cimino et al., 1992; Han et al., 2000), these results may suggest that the primary presynaptic nicotinic receptor population is one that contains the α6 nicotinic receptor subunit. The β subunit present in combination with the α6 subunit in α-conotoxin MII-sensitive nAChRs is also under investigation. Studies with knockout mice show that β2 and β3 subunits are necessary for the majority of α-conotoxin MII binding in rodent striatum (Cordero-Erausquin et al., 2000; Grady et al., 2001). Oocyte expression with combinations of α3, β2, and β3 or α6, β2, and β4 subunits indicates that α-conotoxin MII can bind to nAChRs containing combinations of α3/β2/β3 or α6/β2/β4 subunits (Kuryatov et al., 2000; McIntosh et al., 2000). Therefore, the α-conotoxin MII-sensitive ^{125}I-epibatidine sites (~50%) in the primate caudate-putamen may contain α6, possibly in combination with β2, β3, or β4 nAChR subunits.

The population of ^{125}I-epibatidine binding sites insensitive to α-conotoxin MII may contain α2, α4, or α5 acetylcholine recognition subunits expressed with β2, β3, and/or β4 nAChR subunits (Quik et al., 2000a; Han et al., 2000). In rodents, the majority of high-affinity [^{3}H]nicotine and [^{3}H]cytisine binding sites contain α4 and β2 subunits (Flores et al., 1991; Davila-Garcia et al., 1997). Nicotine, cytisine, and A85380 have high affinity for α4β2 nAChRs expressed in man, whereas other combinations, such as α2β2, α2β4, and α4β4, with or without α5, have marked decreases in affinities for these ligands (Ramirez-Latorre et al., 1996). Based on these findings, it is possible that the α-conotoxin MII-insensitive

component of ^{125}I-epibatidine binding consists of nAChRs containing at least an $\alpha4\beta2$ interface, with or without $\alpha2$, $\alpha5$, $\beta3$, or $\beta4$.

The relative proportions and ligand affinities of the α-conotoxin MII-sensitive and -insensitive nAChR populations in primate caudate-putamen seem to be different from those previously reported in rodent striatum. In monkey caudate-putamen, receptor binding studies show that ~50% of the ^{125}I-epibatidine sites are α-conotoxin MII-sensitive, whereas in rodent striatum, the α-conotoxin MII-sensitive nAChRs comprise only ~15% of epibatidine binding sites (Whiteaker et al., 2000c). In addition, throughout monkey brain, the nAChR populations sensitive and insensitive to α-conotoxin MII have affinities for cytisine and nicotine in the low nanomolar range. In contrast, the nAChR populations expressed in rodent brain seem to have very different affinities for these ligands, with IC_{50} values of 18 and 481 nM for cytisine and nicotine, respectively (Marks et al., 1998; Whiteaker et al., 2000a,b). It has previously been reported in rodents that [^{3}H]nicotine and [^{3}H]cytisine bind predominantly, if not exclusively, to $\alpha4\beta2$ nAChRs (Flores et al., 1991; Davila-Garcia et al., 1997). In monkeys, the similar affinities that cytisine and nicotine have for the α-conotoxin MII-sensitive and -insensitive nAChRs imply that [^{3}H]nicotine and [^{3}H]cytisine binding in the caudate-putamen of humans (Houghtling et al., 1995; Court et al., 2000) may not exclusively label $\alpha4\beta2$ nAChRs and that the decreases in nAChR binding in PD brains could be due to loss of other nAChR subtypes, such as $\alpha3/\alpha6$-containing nAChRs sensitive to α-conotoxin MII.

The relationship of the nAChR populations expressed in the caudate-putamen to basal ganglia function in primates remains to be investigated. In rodents, activation of α-conotoxin MII-sensitive nAChRs accounts for 40% of nicotine-evoked dopamine release from striatum (Kulak et al., 1997; Kaiser et al., 1998; Grady et al., 2001), although α-conotoxin MII maximally inhibits ~15% of epibatidine binding (Whiteaker et al., 2000c). The results from this work show that in the striatum of monkeys, ~50% of the nAChRs that bind epibatidine are α-conotoxin MII-sensitive. If the α-conotoxin MII-sensitive nAChRs in the caudate-putamen of monkeys are involved in nicotine-evoked dopamine release in a manner similar to rodents, these nAChRs may contribute to the majority of presynaptic nicotine-evoked dopamine release in the caudate-putamen. The results presented here imply that drugs that activate α-conotoxin MII-sensitive nAChRs may represent a novel nAChR population for increasing dopamine release in the caudate-putamen of primates.

Our data in the monkey suggest that the α-conotoxin MII-sensitive nAChR population may represent a target for the

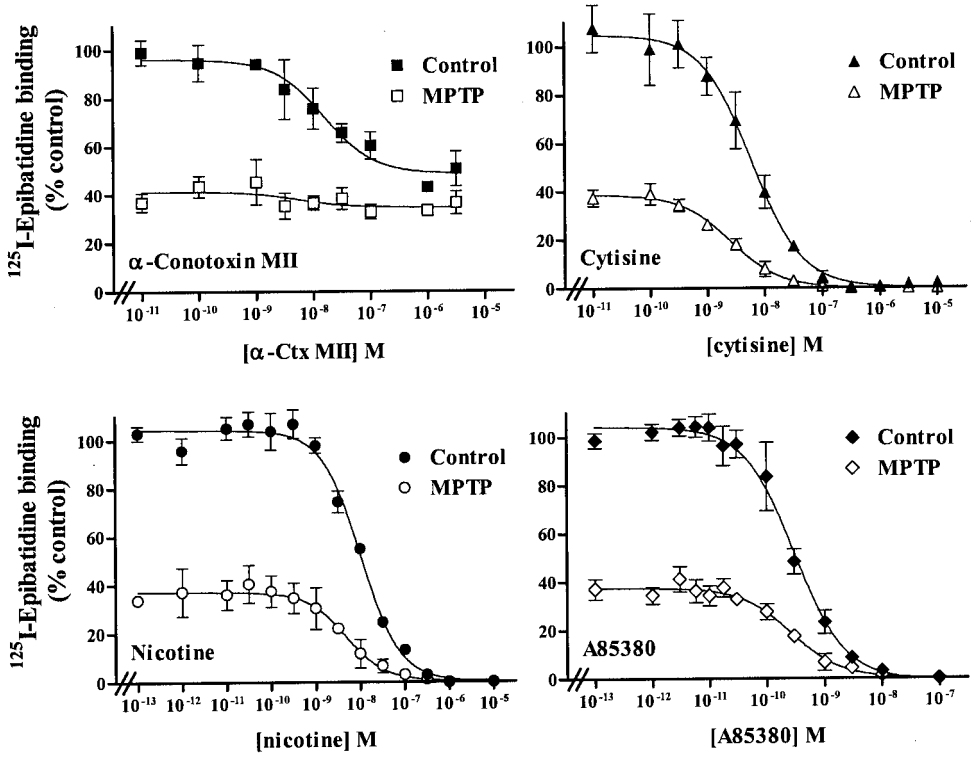

Fig. 7. α-Conotoxin MII-insensitive nAChRs in the caudate of severely lesioned monkeys are sensitive to other nAChR ligands. ^{125}I-Epibatidine inhibition studies were conducted using the nAChR ligands nicotine, cytisine, A85380, and α-conotoxin MII in control and severely lesioned monkeys (MPTP). All curves fit best to a one-site model (see Table 2 for K_i values). Inhibition in putamen was similar. Points are mean ± S.E.M. of three to four experiments. If no error bars are shown, the S.E.M. was within the size of the symbol.

TABLE 3
nAChR populations expressed in monkey caudate-putamen
Subunits are inferred from mRNA expression and sensitivity to nAChR ligands (Quik et al., 2000a,b)

Population	α Subunit	β Subunit	Ligand Sensitivity	Effect of MPTP
				%
α-ctx MII sensitive	$\alpha6$	$\beta2$, $\beta3$, $\beta4$	A85380 > Cyt ≈ Nic > α-ctx MII	100 ↓
α-ctx MII insensitive	$\alpha4$	$\beta2$	A85380 > Cyt ≈ Nic	20 ↓

α-ctx MII, α-conotoxin MII; Nic, nicotine; Cyt, cytisine.

treatment of PD symptoms because these receptors are selectively decreased after nigrostriatal degeneration. However, a question that arises is the therapeutic potential of nicotinic drugs in the presence of substantially reduced receptor expression. Although the receptor sites are greatly reduced in MPTP-treated monkeys, these animals also exhibited 95 to 99% declines in the striatal dopamine transporter. In contrast, striatal dopamine levels are less severely decreased (70 to 80%) in the early stages of PD (Lang and Lozano, 1998), with a potentially greater number of residual α-conotoxin MII-sensitive nAChR. Postmortem studies with brains from individuals with PD are essential to clarify these issues.

Neuronal nAChRs may also represent a target for neuroprotective strategies to halt disease progression. Considerable epidemiological evidence demonstrates that cigarette smokers have a decreased risk for PD (Morens et al., 1995). The mechanism whereby smoking protects against nigrostriatal degeneration is not yet known, although animal studies indicated that nicotine is a potential candidate (Quik and Jeyarasasingam, 2000; Balfour and Fagerstrom, 1996). Considering the fact that PD symptoms develop in humans when 20 to 40% of dopamine levels remain, in contrast to monkeys, agonists directed to nAChRs containing α3/α6 or α4β2 subunits may have the potential for neuroprotective benefits.

In summary, these studies indicate that monkey striatum expresses multiple nAChR populations discriminated by α-conotoxin MII but not nicotine, cytisine, or A85380 and that the decrease in ^{125}I-epibatidine binding in the caudate-putamen of parkinsonian monkeys is due to a loss of α-conotoxin MII-sensitive nAChRs. It has previously been shown that in Parkinson's disease there is a decrease in nAChR binding in the striatum but no decline in α3, α4, α7, or β2 immunoreactivity (Martin-Ruiz et al., 2000; Perry et al., 2000). α6-Containing nAChRs are primarily affected by nigrostriatal lesioning and severe damage is necessary to see an effect upon α4β2-containing nAChRs. Thus, in the early stages of PD, ligands directed toward α6-containing nAChRs (α-conotoxin MII-sensitive) may be important for therapeutics, whereas nAChR ligands with a wider spectrum of activities may be more relevant for advanced PD.

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