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L-DOPA Treatment Modulates Nicotinic Receptors in Monkey Striatum

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ABSTRACT

Nicotinic acetylcholine receptor (nAChR) activation is well known to stimulate dopamine release in the striatum. This phenomenon may be physiologically significant in the control of motor function, as well as in pathological conditions such as Parkinson's disease. An understanding of the mechanisms that influence nAChR expression and function is therefore important. Because the dopamine precursor L-DOPA is the most commonly used therapeutic agent for Parkinson's disease, we investigated the effects of L-DOPA treatment on striatal nAChR expression in unlesioned and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned monkeys. In unlesioned animals, L-DOPA (15 mg/kg) administered twice daily for 2 weeks decreased both ^{125}I-epibatidine and [^{125}I]iodo-3-[2(*S*)-azetidinylmethoxy]pyridine (A-85380) binding sites in the caudate and putamen, but did not affect ^{125}I-α-CtxMII sites. α-CtxMII inhibition of striatal ^{125}I-epibatidine and [^{125}I]A-85380 binding with α-CtxMII suggest that there are both high- ($K_i < 0.2$ nM) and low-affinity ($K_i > 100$ nM) α-CtxMII-sensitive sites, as well as α-CtxMII-resistant sites, and that L-DOPA treatment influences only the low-affinity α-CtxMII-sensitive subtype. The L-DOPA effect was selective for striatal nAChRs with no change in cortical sites. Monkeys with severe nigrostriatal damage did not exhibit L-DOPA-induced declines in striatal nAChRs, suggesting that L-DOPA primarily affects nAChRs associated with dopaminergic terminals. In summary, these data show that L-DOPA treatment decreases nAChR expression, in contrast with the well established up-regulation of these sites by chronic nicotine exposure. Furthermore, they demonstrate preferential L-DOPA regulation of a novel low-affinity α-CtxMII-sensitive site. These declines in nAChRs with L-DOPA may be relevant to both the therapeutic and side effect profiles of L-DOPA therapy in Parkinson's disease.

Parkinson's disease is a movement disorder characterized by a selective loss of nigrostriatal dopaminergic neurons, with marked declines in dopamine levels in the striatum. Knowledge of these neurochemical deficits led to the therapeutic use of the dopamine precursor L-DOPA, which is currently one of the principal agents used for Parkinson's disease therapy (Bezard et al., 2001).

A variety of neurotransmitter systems regulate striatal dopaminergic function, including glutamatergic inputs from the cortex (Roberts and Anderson, 1979; Desce et al., 1991) and possibly serotonergic afferents from the raphe nuclei (Barnes and Sharp, 1999). Acetylcholine released from cholinergic interneurons is also an important modulating factor through activation of nAChRs (Zhou et al., 2001). Mammalian neuronal nAChRs are heterogeneous and seem to be composed of multiple α (α2–α7) and β (β2–β4) subunits (Lukas et al., 1999). To date, several subtypes have been linked to striatal dopaminergic function, including nAChRs containing α4, α6, and β3 subunits in combination with β2 (Wonnacott, 1997; Quik et al., 2000; Grady et al., 2001; Champtiaux et al., 2002; Whiteaker et al., 2002; Zoli et al., 2002).

Because nAChRs influence striatal dopaminergic activity, knowledge of the conditions that affect the different nAChR subtypes is critical. Studies to date have shown that nigrostriatal damage decreases striatal nAChR densities in mice, rats, monkeys, and humans, with a particular vulnerability of the ^{125}I-α-CtxMII-binding population, thought to correspond to α6*[1] nAChRs (Schwartz et al., 1984; Clarke and Pert, 1985; Court et al., 2000; Quik et al., 2001; Zoli et al., 2002; Quik et al., 2003). Cholinergic pharmacological treat-

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[1] The asterisk denotes nicotinic receptors containing the indicated α and/or β subunit and possibly also additional undefined subunits.

ABBREVIATIONS: nAChR, nicotinic acetylcholine receptor; α-CtxMII, α-conotoxin MII; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; A-85380, 3-[2(*S*)-azetidinylmethoxy]pyridine; ANOVA, analysis of variance; [^{125}I]RTI-121, 3β-(4-[^{125}I]iodophenyl)tropane-2β-carboxylic acid isopropyl ester.

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ments are also known to modulate nAChR expression. Prolonged administration of the cholinesterase inhibitor diisopropyl fluorophosphate results in decreased nAChR expression (Schwartz and Kellar, 1985). In contrast, chronic nicotinic agonist treatment up-regulates striatal nAChR expression, a phenomenon often accompanied by decreased nAChR activity (Schwartz and Kellar, 1985; Marks et al., 1992; Collins et al., 1994), although a few examples of enhanced nAChR responsiveness have also been reported (Rowell and Wonnacott, 1990). nAChR up-regulation in the striatum is also observed after nAChR antagonist administration, suggesting that the increased nAChR numbers may be caused by a blockade or desensitization (Collins et al., 1994).

The objective of the present experiments was to determine whether the commonly used Parkinson's disease drug L-DOPA (a compound with dopaminergic, rather than cholinergic activity) influenced striatal nAChR expression. Because nAChR stimulation evokes release of dopamine, there is the possibility of feedback regulation. The present results show that chronic dopamine precursor administration differentially decreases expression of nAChR subtypes. These data suggest that the dopaminergic system can exert a negative modulatory influence on striatal nAChR expression.

Materials and Methods

Animals. Squirrel monkeys (*Saimiri sciureus*) of either sex were purchased from Osage Research Primates (Osage Beach, MO). Animals (0.5–0.8 kg) were housed in a room with a 13:11-h light/dark cycle. Immediately after arrival, the monkeys were quarantined and tested according to standard veterinary practice. The monkeys had free access to water and were given food once daily. All procedures used conform to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

Treatments and Behavioral Evaluation. After an initial acclimatization period, the monkeys were randomly assigned to treatment with saline or MPTP (2 mg/kg s.c.). At 2.5 to 3 weeks after saline or MPTP injection, animals were rated for parkinsonism using a modified Parkinson rating scale for the squirrel monkey (Langston et al., 2000). The disability scores ranged from 0 to a maximum of 20 for a severely parkinsonian animal. The composite score was evaluated by an assessment of 1) spatial hypokinesia (reduction in use of the available cage space), 2) body bradykinesia (increased slowness in body movement), 3) manual dexterity, 4) balance, and 5) freezing. Each parameter was evaluated using a 5-point range with 0 being normal. If the total Parkinson score was less than 6, MPTP treatment was repeated using a lower dose (1.75 mg/kg s.c.) than the first because our previous studies indicated that readministration of 2 mg/kg occasionally (<5%) led to animal mortality. Parkinsonism was evaluated 2.5 to 3 weeks after every MPTP injection treatment, as described above. MPTP administration was repeated up to a maximum of six times in some of the monkeys, until stably parkinsonian. Four to 6 weeks after the last saline or MPTP injection, the animals were administered L-DOPA (15 mg/kg) in combination with the peripheral DOPA decarboxylase inhibitor carbidopa by oral gavage twice daily 4 h apart. This was done using a 5 or 6 day on, 2 or 3 day off and 5 or 6 day on schedule. The animals were then euthanized either 3 h or 3 day after administration of the last dose of L-DOPA, in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and conforming to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Ketamine hydrochloride (15–20 mg/kg i.m.) was administered for sedation, followed by injection of 0.22 ml/kg i.v. euthanasia solution (390 mg of sodium pentobarbital and 50 mg of phenytoin sodium/ml). The brains were then removed, divided along the midline, one-half placed in a mold and cut into 6-mm-thick blocks. These were frozen in isopentane on dry ice and stored at −80°C.

Autoradiographic Studies. Tissue preparation for autoradiography. Brain sections (20 μm) were cut at −15°C using a cryostat (Leica Microsystems, Inc., Deerfield, IL). After thaw mounting onto poly-L-lysine–coated slides, the sections were air dried and stored at −80°C.

[^{125}I]RTI-121 Autoradiography. [^{125}I]RTI-121 (2,200 Ci/mmol; PerkinElmer Life Sciences, Boston, MA) was used to measure binding to the dopamine transporter (Quik et al., 2001). Thawed brain sections were preincubated for 2 × 15 min in 50 mM Tris-HCl buffer, pH 7.4, containing 120 mM NaCl and 5 mM KCl. Incubation (2 h) was initiated using the same buffer plus 0.025% bovine serum albumin (BSA), 1 μM fluoxetine, and 50 pM [^{125}I]RTI-121, in the absence or presence of 100 μM nomifensine to define nonspecific binding. The sections were washed for 4 × 15 min at 4°C in preincubation buffer, dipped in ice-cold water, air-dried, and placed against Kodak MR film (PerkinElmer Life Sciences) for 1 to 3 days with ^{125}I microscale standards (Amersham Biosciences Inc., Piscataway, NJ).

^{125}I-Epibatidine Autoradiography. Binding of ^{125}I-epibatidine (2,200 Ci/mmol; PerkinElmer Life Sciences) was done as described previously (Kulak et al., 2002a). Incubation was at room temperature for 40 min in 50 mM Tris buffer, pH 7.0, containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, and ^{125}I-epibatidine (0.02 nM). Sections were next washed (4°C) twice for 5 min with buffer and once for 10 s in deionized H_2O (4°C). After air-drying, slides were exposed for 2 to 5 days to Kodak MR film, together with ^{125}I standards. Nonspecific binding, defined in the presence of 10^{-4} M nicotine, was the same as the film blank.

[^{125}I]A-85380 Autoradiography. [^{125}I]A-85380 (1450 Ci/mmol) was prepared as described previously (Musachio et al., 1999) and binding to brain sections done as detailed previously (Kulak et al., 2002b). Sections were incubated for 60 min with [^{125}I]A-85380 (0.2 nM) in the same buffer used for the ^{125}I-epibatidine binding assays. Sections were washed in buffer at 4°C for 2 × 5 min and 1 × 10 s in deionized H_2O (4°C). Slides were dried at room temperature and then exposed to Kodak MR film for 1 to 2 days simultaneously with ^{125}I standards. To determine nonspecific binding, sections were also exposed to 10^{-4} M nicotine; blanks were indistinguishable from film background.

^{125}I-α-CtxMII Autoradiography. α-CtxMII was iodinated and receptor autoradiography done as described previously (Quik et al., 2001). Thawed sections were preincubated at room temperature for 15 min in 20 mM HEPES buffer, pH 7.5, containing 144 mM NaCl, 1.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgSO_4$, and also 0.1% BSA and 1 mM phenylmethylsulfonyl fluoride. This was followed by a 1-h incubation with ^{125}I-α-CtxMII (0.5 nM) at room temperature in the same HEPES salt buffer but now also containing 0.5% BSA, 5 mM EDTA, 5 mM EGTA, and 10 μg/ml each of aprotinin, leupeptin, and pepstatin A, rather than 0.1% BSA and 1 mM phenylmethylsulfonyl fluoride. Slides were washed for 30 s in the HEPES salt buffer at room temperature, 30 s in ice-cold buffer, 2 × 5 s in 0.1× buffer (0°C), and 2 × 5 s at 0°C in deionized water. Nonspecific ^{125}I-α-CtxMII binding was defined using 0.1 μM epibatidine. The sections were then air dried and apposed to Kodak MR film for 2 to 5 days simultaneously with ^{125}I standards.

Analysis of Autoradiographic Data. Quantitation of optical densities associated with various brain regions was done using the ImageQuant system from Amersham Biosciences Inc. The optical density values were converted to femtomoles per milligram of tissue using standard curves generated from ^{125}I standards simultaneously exposed to the films. Specific binding was determined by subtracting background from total values. Activity levels of the autoradiographic ^{125}I standards ranged from 0.5 to 200 nCi/mg tissue for 20-μm-thick tissue samples as per the manufacturer's specifications (Amersham Biosciences Inc.). Sample optical density readings were within the

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linear range of the film. The receptor binding value for a brain area for any one animal was obtained from two independent experiments, with one or two consecutive sections per experiment. All values are expressed as the mean ± S.E.M. of the indicated number of animals. Statistical analyses were done using either Student's paired t test, or one-way ANOVA followed by Newman-Keuls multiple comparison test where $p \leq 0.05$ was considered significant.

Receptor Studies Using Membrane Preparations. Tissue preparation for membrane binding assays. Monkey striatal tissue was dissected from thawed 6-mm-thick blocks. Striatal samples were homogenized in 2× physiological buffer, pH 7.5 (288 mM NaCl, 4 mM KCl, 4 mM $CaCl_2$, 2 mM $MgSO_4$, and 40 mM HEPES; 22°C) supplemented with phenylmethylsulfonyl fluoride (100 μM), using a glass-Teflon tissue grinder. Homogenates were allowed to incubate with the phenylmethylsulfonyl fluoride (15 min, 22°C) to inactivate endogenous serine proteases and then particulate fractions were obtained by centrifugation (20,000g, 20 min, 4°C; Sorval RC-2B centrifuge). The pellets were resuspended in distilled, deionized water, incubated for 10 min at 22°C, and then harvested by centrifugation as before. Each pellet was washed twice more with distilled, deionized water by resuspension/centrifugation, and then stored (in pellet form under 0.1× physiological buffer) at −70°C until used.

Simultaneous Determination of α-CtxMII-Sensitive and -Resistant ^{125}I-Epibatidine Saturation Binding to Striatal Membranes. The densities and α-CtxMII sensitivities of the ^{125}I-epibatidine binding populations expressed in monkey striatum were measured using a similar approach to that described by Whiteaker et al. (2000). Incubations were performed in 96-well siliconized polypropylene plates, in 30 μl of protease inhibitor buffer [physiological buffer supplemented with bovine serum albumin, 0.1% (w/v); 5 mM EDTA, 5 mM EGTA, and 10 μg/ml each of aprotinin, leupeptin trifluoroacetate, and pepstatin A]. Plates were covered to minimize evaporation during incubation, and all experiments were incubated for 3 h at 22°C. Saturation binding experiments were performed in duplicate using ^{125}I-epibatidine concentrations ranging between 5 and 400 pM. At each concentration of ^{125}I-epibatidine, inhibition of specific ligand binding by α-CtxMII was determined, in duplicate, at a range of α-CtxMII concentrations. This allowed the amounts of each α-CtxMII-sensitive or -resistant ^{125}I-epibatidine binding population to be determined at each radioligand concentration. Binding reactions were terminated by filtration of samples onto a single thickness of polyethyleneimine-soaked [0.5% (w/v) in physiological buffer] GF/F filter paper (Whatman Inc., Clifton, NJ), using a cell harvester (Inotech, Rockville, MD). Samples were subsequently washed six times with ice-cold physiological buffer. Total and nonspecific [in the presence of 1 mM (−)-nicotine tartrate] binding were determined in duplicate for each ^{125}I-epibatidine binding concentration. Bound ligand was quantified by gamma counting at 83 to 85% efficiency and converted to femtomoles per milligram of protein using data from the protein assay. At the lower ligand concentrations, a significant proportion of ligand bound to the tissue. Free ^{125}I-epibatidine concentrations were calculated by correcting for the amount of ligand bound to the tissue. These corrected concentrations were then used to perform independent saturation analyses for each (α-CtxMII-sensitive or -resistant) ^{125}I-epibatidine population detected.

Simultaneous Determination of α-CtxMII-Sensitive and -Resistant [^{125}I]A-85380 Saturation Binding to Striatal Membranes. The techniques used were identical to those described for ^{125}I-epibatidine binding (see above), except that saturation binding was performed using [^{125}I]A-85380 concentrations ranging from approximately 5 to 400 pM.

α-CtxMII Inhibition of ^{125}I-Epibatidine Binding to Striatal Membranes. Inhibition binding experiments were performed in triplicate using 400 pM ^{125}I-epibatidine (equivalent to 1000 Bq/well, and a saturating concentration, ensuring accurate quantitation of each striatal ^{125}I-epibatidine binding population). The amount of membrane protein added was chosen to produce maximum ligand binding to the tissue of approximately 80 Bq/well (<10% of total ligand added, minimizing the effects of ligand depletion). The densities of α-CtxMII-sensitive and -resistant ^{125}I-epibatidine binding populations were determined by inhibition with α-CtxMII (0.1–3,000 nM). Total and nonspecific [in the presence of 1 mM (−)-nicotine tartrate] binding were determined for each striatal sample. All determinations were performed in triplicate.

Data Analysis of Membrane Binding Studies. Results for inhibition binding by α-CtxMII were fit using a three-site fit: $B = B_1/(1 + (I/IC_{50-1})) + B_2/(1 + (I/IC_{50-2})) + B_3$, where B is ligand bound at inhibitor concentration I; B_1 and B_2 are binding sites sensitive to α-CtxMII inhibition with IC_{50-1} and IC_{50-2}, respectively; and B_3 represents α-CtxMII-resistant binding sites. ^{125}I-Epibatidine and [^{125}I]A-85380 saturation binding parameters were calculated for the sites represented by B_1, B_2, and B_3, using the Hill equation: $B = B_{max} L^{nH}/(L^{nH} + K_D{}^{nH})$, where B is the binding at the free ligand concentration L, B_{max} is the maximum number of binding sites, K_D is the equilibrium binding constant, and n_H is the Hill coefficient. Values of B_{max}, K_D, and n_H were calculated using the nonlinear least-squares fitting algorithm of SigmaPlot 2001 (Jandel Scientific, San Rafael, CA). Values of K_i (inhibition binding constant) were derived for the sites represented by B_1 and B_2, for both ^{125}I-epibatidine and [^{125}I]A-85380, using the method of Cheng and Prusoff (1973): $K_i = IC_{50}/1 + (L/K_D)$. Statistical analyses were performed where indicated, using one-way ANOVA.

Results

Effect of Drug Treatments on Animal Behavior and the Nigrostriatal Dopaminergic System. Animals treated with the dopaminergic neurotoxin MPTP developed motor deficits characteristic of Parkinson's disease. These included bradykinesia, hypokinesia, freezing, loss of balance, and/or decreased manual dexterity. The Parkinson scores, before water or L-DOPA gavage, were 6.5 ± 1.1 and 8.9 ± 2.0,

TABLE 1

Parkinson rating scores in control and MPTP-lesioned monkeys treated without and with L-DOPA

After an initial acclimatization period, the monkeys were rated for parkinsonism daily over a 1-to-2 week period using a Parkinson rating scale modified for the squirrel monkey. The animals then received several saline or MPTP (1.5 to 2 mg/kg s.c.) injections. Four weeks or more after the last treatment, parkinsonism was again evaluated daily over a 2-week period. The animals were subsequently gavaged with water or L-DOPA (15 mg/kg twice daily for 1 to 2 weeks) and parkinsonism rated during the course of the L-DOPA treatment. Each value represents the mean ± S.E.M. of the average Parkinson score for each monkey.

Monkey Group	No. of Animals	MPTP Treatment	L-DOPA Treatment	Parkinson Rating Score	
				No L-DOPA	L-DOPA
Control	10	−	−	0.1 ± 0.1	NA
	10	−	+	0	NA
MPTP	8	+	−	6.5 ± 1.1*	NA
	6	+	+	8.9 ± 2.0*	4.5 ± 1.3**

NA, not available.
* p <0.001, significantly different from control using a one-way ANOVA.
** p < 0.01, significantly different from MPTP alone using a paired t test.

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respectively (Table 1). To determine the effectiveness of L-DOPA treatment, Parkinson rating scores were evaluated 2 to 3 h after L-DOPA administration, a time at which the effect of the drug is maximal. L-DOPA treatment significantly ($p < 0.01$) reversed the motor abnormalities by ~50%, with a decrease in the rating from 8.9 ± 2.0 to 4.5 ± 1.3, consistent with previous results (Langston et al., 2000).

Dopamine transporter autoradiography was done as a biochemical measure of MPTP-induced nigrostriatal damage (Fig. 1). Binding of [^{125}I]RTI-121 (50 pM) to control monkey brain, in femtomoles per milligram of tissue, was as follows: medial caudate, 15.1 ± 1.1 (n = 10); lateral caudate, 12.2 ± 0.7 (n = 10); ventromedial putamen, 13.9 ± 1.3 (n = 10); ventrolateral putamen, 12.2 ± 0.8 (n = 10); and dorsal putamen, 10.8 ± 0.9 (n = 10). After MPTP treatment, an 80 to 90% decline was observed in the medial and lateral caudate, and ventrolateral and dorsal putamen with a somewhat smaller decline (60%) in the ventromedial putamen. The MPTP-treated animals in the present study are thus similar to those previously defined as severely lesioned (Quik et al., 2001; Kulak et al., 2002a). L-DOPA treatment had little effect on dopamine transporter levels in either the unlesioned or lesioned animals, except for a small (~10%), but significant decline in transporter levels in the lateral caudate in unlesioned animals.

Nicotinic Autoradiographic Studies. The effects of L-DOPA treatment on striatal nAChR populations in unlesioned animals. The effects of L-DOPA treatment on striatal nicotinic binding populations were investigated using quantitative autoradiography. Three radioligands were used in these studies, including ^{125}I-epibatidine, [^{125}I]A-85380, and ^{125}I-α-CtxMII. ^{125}I-Epibatidine binds to a wide range of neuronal nAChR subtypes (Whiteaker et al., 2000; Kulak et al., 2002a; Whiteaker et al., 2002) and thus provides a broad overview of nAChR expression. [^{125}I]A-85380 binds to a more restricted subset of neuronal nAChRs than ^{125}I-epibatidine, those that contain the β2 subunit (Kulak et al., 2002a,b; Perry et al., 2002). Finally, ^{125}I-α-CtxMII was chosen because it interacts with an even more restricted set of neuronal nAChRs (thought to represent $\alpha6\beta2$*; Champtiaux et al., 2002; Whiteaker et al., 2002; Zoli et al., 2002) that are preferentially expressed on dopamine terminals in monkey striatum (Quik et al., 2001).

^{125}I-Epibatidine autoradiographic studies were first done to determine the effect of L-DOPA treatment in the caudate and putamen (Figs. 2, 3, and 4). Binding of ^{125}I-epibatidine (0.02 nM) to control monkey brain, in femtomoles per milligram of tissue, was as follows: medial caudate, 3.03 ± 0.11 (n = 15); lateral caudate, 2.40 ± 0.09 (n = 15); ventromedial putamen, 3.15 ± 0.08 (n = 15); ventrolateral putamen, 2.76 ± 0.09 (n = 15); dorsal putamen, 2.43 ± 0.08 (n = 15). L-DOPA administration resulted in significant reductions in ^{125}I-epibatidine sites in unlesioned animals with ~20% decreases in the medial and lateral caudate and 15 to 20% declines in the different putamen areas (Figs. 3 and 4).

We next investigated the effect of L-DOPA treatment on striatal [^{125}I]A-85380 binding sites using autoradiography (Figs. 2–4). Binding of [^{125}]A-85380 (0.20 nM) to control monkey brain, in femtomoles per milligram of tissue, was as

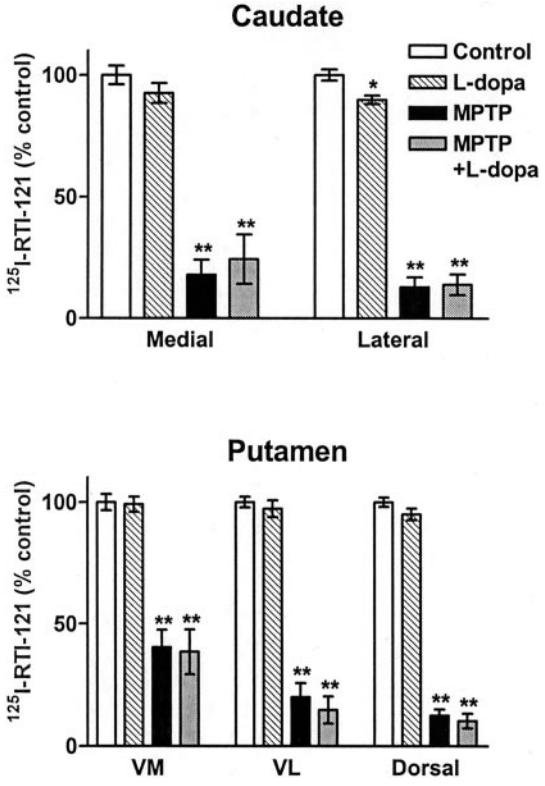

Fig. 1. Dopamine transporter binding to monkey striatum after L-DOPA and/or MPTP treatments. Monkeys were treated with saline or MPTP and subsequently administered water or L-DOPA as described under *Materials and Methods*. Dopamine transporter binding to monkey caudate and putamen was determined using [^{125}I]RTI-121. VL, ventrolateral, VM, ventromedial. Each column represents the mean ± S.E.M. of 6 to 10 animals. Significance of difference from control, *, $p < 0.01$; **, $p < 0.001$.

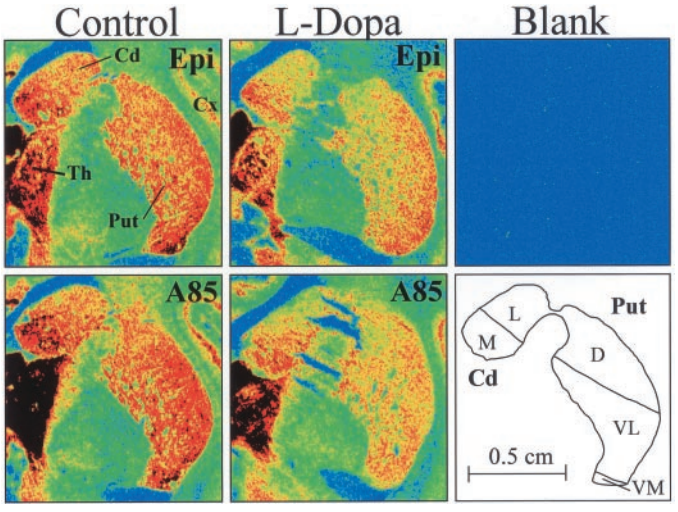

Fig. 2. Computer generated autoradiograms demonstrating the effect of L-DOPA treatment on nAChR binding to caudate and putamen from unlesioned animals. Monkeys were administered water or L-DOPA (15 mg/kg twice daily for 2 weeks) as described under *Materials and Methods*. ^{125}I-Epibatidine (Epi) binding is depicted at the top and [^{125}I]A-85380 (A85) binding at the bottom. Note the decline in binding with L-DOPA treatment. Nonspecific binding (Blank) was the same using either ^{125}I-epibatidine or [^{125}I]A-85380 (film blank). The images for any particular radioligand are standardized to each other, although the control images using the different ligands are not because the experiments were done independently. Image intensity increases in the following color sequence: blue, yellow, red, and black. Cd, caudate; Cx, cortex; D, dorsal; L, lateral; M, medial; Put, putamen; Th, thalamus; VL, ventrolateral; VM, ventromedial. Scale, 0.5 cm, depicted in the lower left-hand corner of the diagrammatic representation of the striatum.

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follows: medial caudate, 9.07 ± 0.36 (n = 9); lateral caudate, 7.85 ± 0.45 (n = 9); ventromedial putamen, 8.32 ± 0.30 (n = 9); ventrolateral putamen, 8.17 ± 0.31 (n = 9); and dorsal putamen, 7.68 ± 0.43 (n = 9). In unlesioned animals (Figs. 3 and 4), L-DOPA treatment significantly reduced striatal [^{125}I]A-85380 sites with an ~25% decrease in the medial and lateral caudate and ~20% decline in the different putamen areas.

Autoradiography experiments were subsequently done to measure the effects of L-DOPA on striatal ^{125}I-α-CtxMII nAChRs (Figs. 3 and 4). ^{125}I-α-CtxMII (0.5 nM) binding in control monkey brain was as follows (in femtomoles per milligram of tissue): medial caudate, 4.13 ± 0.37 (n = 14); lateral caudate, 2.98 ± 0.27 (n = 14); ventromedial putamen, 3.32 ± 0.35 (n = 14); ventrolateral putamen, 3.15 ± 0.32 (n = 14); and dorsal putamen, 3.06 ± 0.32 (n = 14). L-DOPA treatment did not alter ^{125}I-α-CtxMII binding in any region of the caudate or putamen in unlesioned animals.

Effects of L-DOPA Treatment on Striatal Nicotinic Binding Populations in MPTP-Lesioned Animals. L-DOPA is routinely used in the treatment of Parkinson's disease, and MPTP-lesioning is the most commonly used experimental model of Parkinson's disease. In addition, MPTP lesions preferentially ablate ^{125}I-α-CtxMII binding nAChRs in monkey striatum, an effect thought to reflect expression of these nAChRs on the dopaminergic terminals targeted by MPTP (Quik et al., 2001). These combined findings led us to investigate the effects of L-DOPA treatment on nicotinic binding populations in MPTP-lesioned animals.

The effect of L-DOPA treatment on the distribution of ^{125}I-epibatidine binding sites in animals with nigrostriatal damage was evaluated using autoradiography (Figs. 3 and 4). MPTP lesioning reduced striatal ^{125}I-epibatidine binding by ~50 to 60%. In contrast to the results with unlesioned animals, L-DOPA treatment did not affect ^{125}I-epibatidine binding.

The results obtained using [^{125}I]A-85380 autoradiography were similar to those collected using ^{125}I-epibatidine. MPTP treatment decreased striatal [^{125}I]A-85380 binding sites by 60 to 70%, and again the sites remaining after nigrostriatal damage were insensitive to modulation by L-DOPA treatment (Figs. 3 and 4).

In agreement with our previous findings (Quik et al., 2001), large declines (>95%) were observed in the autoradiographic distribution of ^{125}I-α-CtxMII binding sites in monkeys with severe nigrostriatal damage compared with control (Figs. 3 and 4). Perhaps unsurprisingly given that MPTP treatment essentially abolished striatal ^{125}I-α-CtxMII binding sites, L-DOPA treatment had no significant effect on the expression of these sites in lesioned animals.

L-DOPA Treatment Selectively Regulates ^{125}I-Epibatidine and [^{125}I]A-85380 Binding Sites in the Striatum, but not Cortex. To determine whether the L-DOPA-induced decrease in nAChR binding was restricted to striatal areas, ^{125}I-epibatidine and [^{125}I]A-85380 sites were assessed using autoradiography in the cortex of unlesioned and MPTP-lesioned monkeys treated without and with L-DOPA. There was no change in the binding of either radioligand to the cortex of monkeys under any condition, or combination of conditions, despite significant declines in radioligand binding in the caudate and putamen of animals treated with either MPTP and L-DOPA (Table 2).

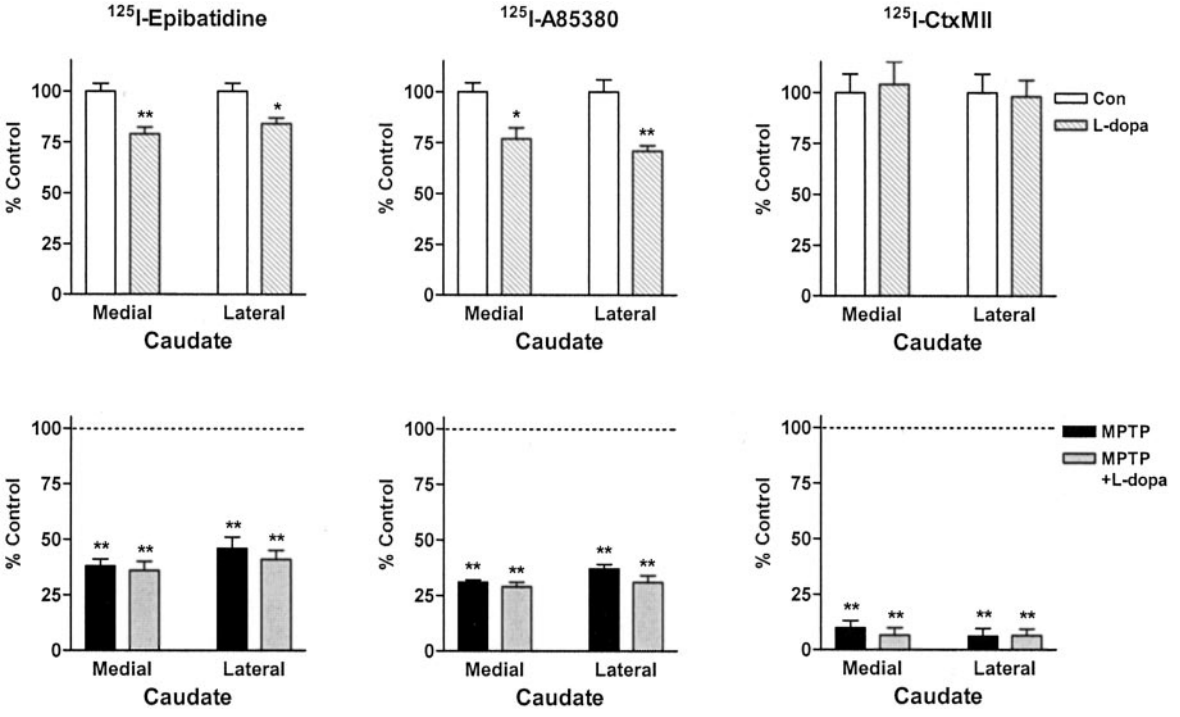

Fig. 3. Quantitative autoradiography of ^{125}I-epibatidine, [^{125}I]A-85380, and ^{125}I-α-CtxMII binding in the caudate nucleus of control (top) and MPTP-lesioned monkeys (bottom) after L-DOPA treatment. Note that L-DOPA treatment results in a decrease in both ^{125}I-epibatidine and [^{125}I]A-85380 binding, but no change in ^{125}I-α-CtxMII binding in unlesioned animals. These data suggest that L-DOPA treatment does not affect high affinity α-CtxMII-sensitive sites. In contrast to the results in unlesioned animals, nAChRs remaining after severe nigrostriatal damage were unaffected by L-DOPA treatment. The columns represent the mean ± S.E.M. of 9 to 15 control, 8 to 9 L-DOPA-treated animals, 8 to 10 MPTP-lesioned and 5 to 6 MPTP-lesioned L-DOPA-treated animals. Significance of difference from control, *, $p < 0.01$; **, $p < 0.001$.

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L-DOPA-Induced Declines in Striatal ^{125}I-Epibatidine and [^{125}I]A-85380 Binding Sites Persist for at Least 3 Days. For the L-DOPA studies, animals were killed either 3 h or 3 days after L-DOPA administration. Analyses of the autoradiography data for ^{125}I-epibatidine and [^{125}I]A-85380 confirmed L-DOPA treatment-induced decreases in striatal binding relative to control, for both radioligands, at both time points. For instance, control [^{125}I]A-85380 binding in the medial caudate was 9.07 ± 0.36 (n = 9), and binding in the 3 h and 3 days post-L-DOPA groups was 6.81 ± 0.43 (n = 4) and 6.92 ± 0.90 (n = 3) fmol/mg, respectively ($p < 0.05$ at both 3 h and 3 days, compared with control using one-way ANOVA). The data for L-DOPA-treated animals were pooled, because no significant differences were seen between the 3-h and 3-day post-L-DOPA groups. The observation that L-DOPA-induced declines in nAChRs persist at least 3 days after its administration is consistent with our behavioral data indicating that the effects of L-DOPA (that is, the development of L-DOPA-induced dyskinesias) may persist several days after treatment (M. Quik, D. Togasaki, J. W. Langstone, D. Di Monte, unpublished observation).

Receptor Studies Using Membrane Preparations. Saturation analysis of ^{125}I-epibatidine and [^{125}I]A-85380 binding in the presence of α-CtxMII indicates that the two radioligands label the same populations of striatal sites. The autoradiography results show that L-DOPA administration modulates striatal ^{125}I-epibatidine and [^{125}I]A-85380 binding sites in unlesioned animals, whereas ^{125}I-α-CtxMII bind-

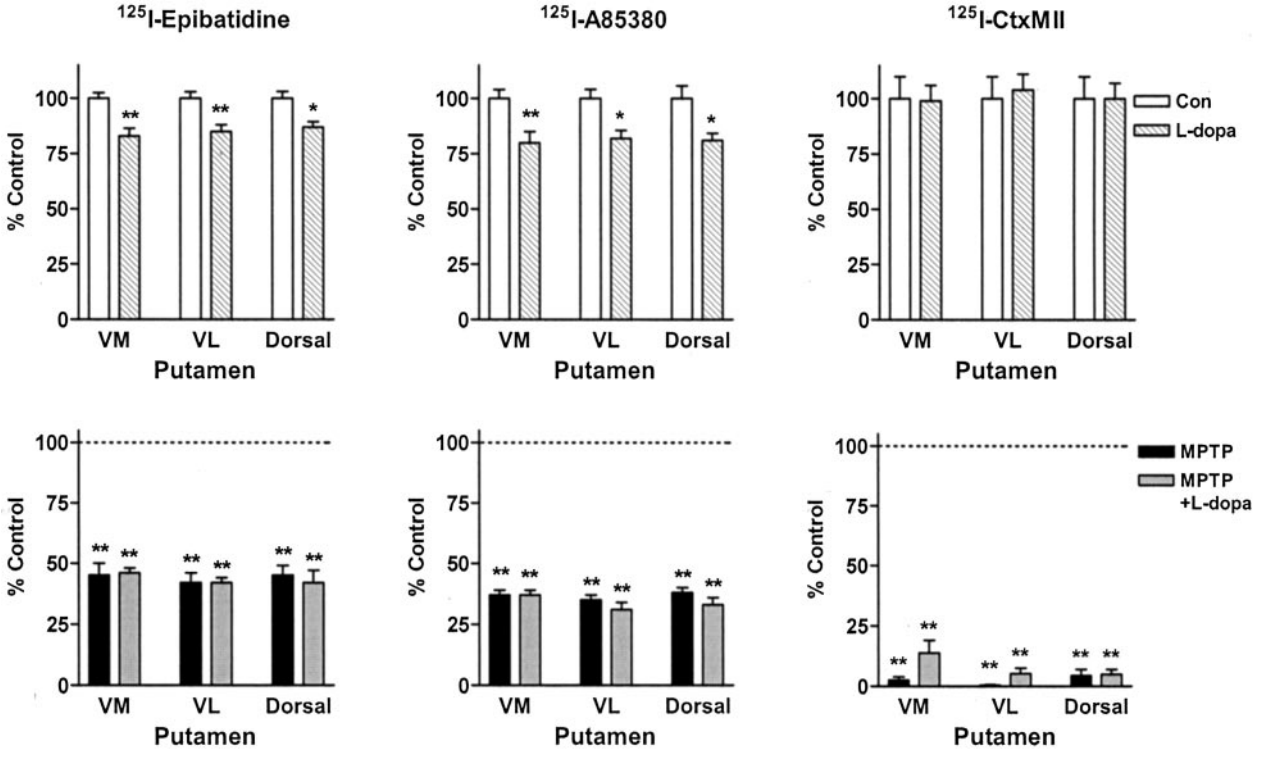

Fig. 4. Binding of ^{125}I-epibatidine, [^{125}I]A-85380, and ^{125}I-α-CtxMII in the putamen of control (top) and MPTP-lesioned monkeys (bottom) after L-DOPA treatment. Quantitative autoradiography shows that L-DOPA treatment results in a decrease in both ^{125}I-epibatidine and [^{125}I]A-85380 binding, but no change in ^{125}I-α-CtxMII binding in unlesioned animals, again suggesting no effect of L-DOPA treatment on high affinity α-CtxMII-sensitive sites. As in the caudate, nAChRs remaining after severe nigrostriatal damage were not decreased after L-DOPA administration. The columns represent the mean ± S.E.M. of 9 to 15 control, 8 to 9 L-DOPA-treated animals, 8 to 10 MPTP-lesioned and 5 to 6 MPTP-lesioned L-DOPA-treated animals. Significance of difference from control, *, $p < 0.01$; **, $p < 0.001$.

TABLE 2

L-DOPA treatment selectively decreases striatal nAChRs

Monkeys were treated with saline or MPTP and subsequently administered water or L-DOPA as detailed in the legend to Table 1. Binding of ^{125}I-epibatidine and [^{125}I]A-85380 to tissue sections was done using 0.02 and 0.2 nM radioligands, respectively. The results shown below are for medial caudate, but similar results were observed for the other striatal areas. Each value represents the mean ± S.E.M. of six to nine monkeys.

Region	L-DOPA	MPTP	^{125}I-Epibatidine Binding		[^{125}I]A-85380 Binding	
			fmol/mg of tissue	*% control*	*fmol/mg of tissue*	*% control*
Caudate	−	−	3.03 ± 0.11	100	8.94 ± 0.53	100
	+	−	2.38 ± 0.10*	78	6.88 ± 0.39*	77
	−	+	1.25 ± 0.11*	41	2.87 ± 0.11*	32
	+	+	1.18 ± 0.14*,**	39	2.69 ± 0.22*,**	30
Cortex	−	−	1.71 ± 0.06	100	3.73 ± 0.11	100
	+	−	1.48 ± 0.06	87	3.70 ± 0.18	99
	−	+	1.61 ± 0.14	94	3.63 ± 0.14	97
	+	+	1.62 ± 0.12	95	3.18 ± 0.23	85

* $p < 0.001$, significantly different from nonlesioned animals not receiving L-DOPA.
** $p < 0.001$, significantly different from nonlesioned animals receiving L-DOPA.

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ing sites are not affected by the same treatment. These data suggest that there are multiple striatal nAChR subtypes differentially regulated in response to L-DOPA administration. This possibility was investigated using a membrane binding approach because it is more conducive to detailed pharmacological analyses than quantitative autoradiography.

Simultaneous Determination of α-CtxMII-Sensitive and -Resistant ^{125}I-Epibatidine Saturation Binding to Monkey Striatal Membranes. The densities and α-CtxMII sensitivities of the ^{125}I-epibatidine binding populations expressed in monkey striatum were assessed using a similar approach to that described by Whiteaker et al. (2000). The results in Fig. 5 (top) show that α-CtxMII inhibited ^{125}I-epibatidine binding to unlesioned monkey striatal membranes at each radioligand concentration used. Not all striatal ^{125}I-epibatidine binding sites were sensitive to α-CtxMII inhibition, indicating the presence of a substantial α-CtxMII-resistant nAChR population in this tissue. Importantly, the dose-response curves for α-CtxMII inhibition of ^{125}I-epibatidine binding were distinctly biphasic, which was most easily discerned at high radioligand concentrations (Fig. 5, top). Thus, striatal α-CtxMII-sensitive ^{125}I-epibatidine binding sites may be separated into high- and low-affinity populations. Binding of ^{125}I-epibatidine to the following three sites was measured at each concentration of ^{125}I-epibatidine; high- and low-affinity α-CtxMII-sensitive, and α-CtxMII-resistant sites. Saturation analysis was then performed for these three sites at each concentration of ^{125}I-epibatidine (Fig. 5, middle). The K_D, B_{max}, and n_H values for ^{125}I-epibatidine calculated for each population are summarized in Table 3. The total density of striatal ^{125}I-epibatidine binding sites in monkey (77.5 fmol/mg protein) is somewhat lower than that reported for [^{3}H]epibatidine in mouse striatum (118 fmol/mg protein; Whiteaker et al. (2000), whereas the density of high-affinity α-CtxMII-sensitive sites (14 versus 16 fmol/mg protein) in monkey and mouse striatum, respectively) is very similar. However, monkey striatum also contains a low-affinity α-CtxMII-sensitive population of ^{125}I-epibatidine binding sites that was not detected in mouse striatum. As a result, the overall proportion of α-CtxMII-sensitive ^{125}I-epibatidine binding sites in monkey striatum is much greater than that reported for the rodent model (37 versus 14%, respectively). Importantly, the high- and low-affinity α-CtxMII populations both exhibited somewhat higher ^{125}I-epibatidine affinities than did the α-CtxMII-resistant site. Thus, at low ^{125}I-epibatidine concentrations (such as used in the autoradiography experiments), the apparent proportion of α-CtxMII-sensitive sites expressed in striatal preparations will be somewhat exaggerated.

Simultaneous Determination of α-CtxMII-Sensitive and -Resistant [^{125}I]A-85380 Saturation Binding to Monkey Striatal Membranes. To determine whether ^{125}I-epibatidine (0.02 nM) and [^{125}I]A-85380 (0.20 nM) bound to analogous sets of nicotinic sites in monkey striatum, α-CtxMII interaction with [^{125}I]A-85380 binding sites expressed in monkey striatum were compared with that of the ^{125}I-epibatidine binding sites. Similarly to ^{125}I-epibatidine, [^{125}I]A-85380 binding in striatum could be divided into high- and low-affinity α-CtxMII-sensitive, as well as an α-CtxMII-resistant, populations. Saturation analysis of these populations (Fig. 5, bottom; Table 3)

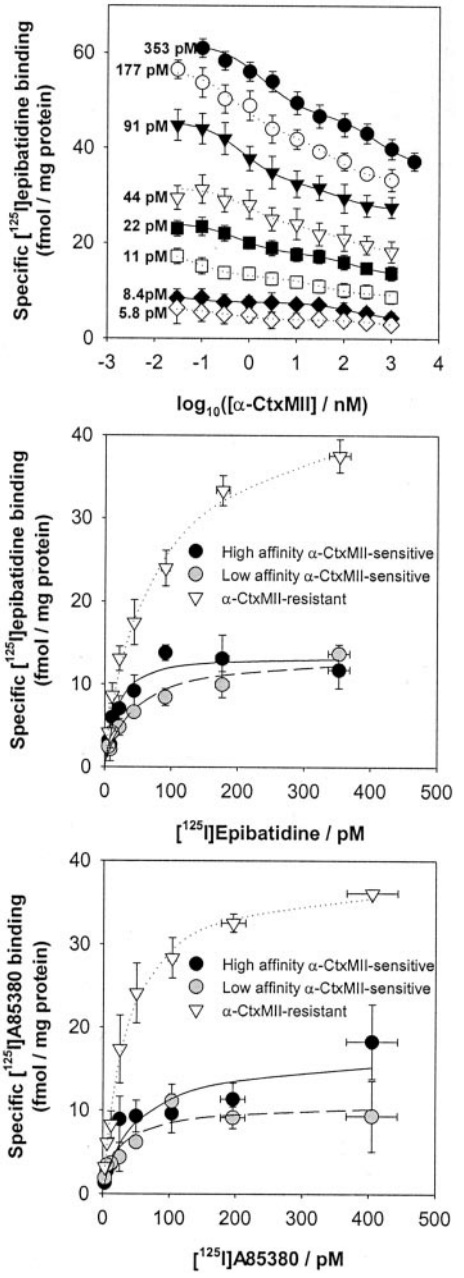

Fig. 5. Simultaneous determination of α-CtxMII-sensitive and -resistant ^{125}I agonist saturation binding to monkey striatal membranes. Top, brain membranes were incubated with ^{125}I-epibatidine (~5–400 pM; indicated on the left of each best fit line) in the presence of varying concentrations of α-CtxMII (0.03–3000 nM). Nonspecific binding was determined by addition of 1 mM (−)-nicotine and was subtracted from total binding to determine specific binding at each combination of ^{125}I-epibatidine and α-CtxMII concentrations. Specific binding was fit to a three-site inhibition binding equation (see *Materials and Methods*), the results of which indicated the densities of the high- and low-affinity α-CtxMII-sensitivity, and the α-CtxMII-resistant ^{125}I-epibatidine binding populations at each concentration of labeled ligand. Middle, the densities of the three ^{125}I-epibatidine binding populations were plotted at each concentration of labeled ligand, and each population was subjected to Hill saturation analysis, providing values of B_{max}, K_D, and n_H for ^{125}I-epibatidine at each site (Table 3). Bottom, α-CtxMII displaced [^{125}I]A-85380 in a similar manner to that seen for ^{125}I-epibatidine. The high- and low-affinity α-CtxMII-sensitive, and α-CtxMII-resistant [^{125}I]A-85380 binding populations found in monkey striatum were quantified in the same way as for ^{125}I-epibatidine binding sites (top). The three phases of [^{125}I]A-85380 binding were plotted at each concentration of radioligand, and were subjected to Hill saturation analysis. The values of B_{max}, K_D, and n_H for [^{125}I]A-85380 binding at each site are summarized in Table 3.

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indicated that they were not significantly different from those measured using ^{125}I-epibatidine (one-way ANOVA). In addition, the K_i values calculated for α-CtxMII at each of the sites were statistically indistinguishable (Table 3, legend). The combination of similar population sizes and α-CtxMII affinities for the three sites measured using ^{125}I-epibatidine and [^{125}I]A-85380 strongly suggests that both radioligands in fact measure very similar, if not identical, populations of nicotinic sites in monkey striatum.

α-CtxMII Inhibition of ^{125}I-Epibatidine Binding Indicates that L-DOPA Treatment Selectively Modulates the Low-Affinity α-CtxMII-Sensitive Population. The effects of L-DOPA treatment on each of the nicotinic sites present in striatum were then investigated using α-CtxMII inhibition of ^{125}I-epibatidine binding. ^{125}I-Epibatidine was chosen for these studies because it had a higher specific activity than [^{125}I]A-85380, thus yielding a larger, more accurately quantifiable signal.

The results of the inhibition binding experiment are presented in Table 4. L-DOPA treatment had no effect on the densities of the high-affinity α-CtxMII-sensitive and α-CtxMII-resistant ^{125}I-epibatidine binding populations, but induced a significant reduction in the density of low-affinity α-CtxMII-sensitive sites [control, 10.1 ± 1.7 fmol/mg protein; L-DOPA-treated, 4.1 ± 0.7 fmol/mg protein; one-way ANOVA, $F(1,6) = 10.21$, $p = 0.0187$]. Thus, as suggested from the autoradiography data, the membrane studies show that L-DOPA treatment exclusively modulates the expression of ^{125}I-epibatidine sites with a lower affinity for α-CtxMII (K_i of ≈100 nM).

Discussion

The present results are the first to show that administration of the dopamine precursor L-DOPA, one of the primary drugs used for the treatment of Parkinson's disease, decreases nAChR expression in the caudate and putamen of normal animals. The dose of L-DOPA used in the current study is within the dosage range used in Parkinson patients (Bezard et al., 2001) and typical of that administered to nonhuman primates to ameliorate MPTP-induced motor deficits (Morissette et al., 1996; Rioux et al., 1997). Receptor sites were evaluated in different areas of the caudate and putamen because of the unique patterns of innervation and/or neuronal cell types in different striatal areas (Kemel et al., 1989; Fearnley and Lees, 1991). In addition, there is a higher density of ^{125}I-α-CtxMII sites in the medial compared with the lateral caudate, possibly suggesting a differential role of these sites. Quantitative autoradiography showed that L-DOPA treatment decreased ^{125}I-epibatidine and [^{125}]A-85380 binding sites, but not high affinity ^{125}I-CtxMII binding, with a somewhat more pronounced effect in the caudate than in the putamen and no regional distinctions within these areas.

The striatum is one of a restricted set of brain regions that expresses α-CtxMII-sensitive nAChRs (Quik et al., 2001; Champtiaux et al., 2002; Whiteaker et al., 2002; Zoli et al., 2002), and striatal ^{125}I-α-CtxMII binding sites are particularly sensitive to nigrostriatal damage (Quik et al., 2001; Kulak et al., 2002a). It was therefore of interest to determine whether α-CtxMII-sensitive nAChRs were modulated by L-DOPA treatment. Autoradiographic analyses showed high-affinity ^{125}I-α-CtxMII sites were unaffected by L-DOPA treat-

TABLE 3

Binding parameters for ^{125}I-epibatidine and [^{125}I]A-85380 at striatal high- and low-affinity α -CtxMII-sensitive, and α -CtxMII-resistant sites

Binding of ^{125}I-epibatidine and [^{125}I]A-85380 to striatal membranes was performed as described in text. Three populations of nicotinic binding sites were identified with each radioligand. These sites had similar affinities for the radioligands but could be distinguished by their distinctly different affinities for α -CtxMII. Each value represents the mean ± S.E.M. of four animals. The K_i values for α -CtxMII inhibition of [^{125}I]epibatidine binding for the high- and low-affinity sites were significantly different from one another (two-way ANOVA, $p < 0.001$), with similar results for [^{125}I]A-85380 binding. No significant differences were detected when the binding densities of each of the sites were compared between the two radioligands (one-way ANOVA, $p > 0.05$). As well, the α -CtxMII K_i values obtained at the high-affinity α -CtxMII–sensitive sites were statistically indistinguishable when determined using either of the radioligands; similar results were obtained for the low-affinity sites.

Ligand	Parameter	α-Ctx-MII–Sensitive Sites		α-Ctx-MII–Resistant Site
		High Affinity	Low Affinity	
^{125}I-Epibatidine	B_{max} (fmol/mg of protein)	14.0 ± 1.9	14.6 ± 2.2	48.9 ± 6.2
	n_H	1.41 ± 0.25	1.07 ± 0.07	0.95 ± 0.06
	K_D (pM)	21.7 ± 5.5	57.5 ± 26.7	88.8 ± 29.6
	K_i for α-CtxMII (nM)	0.15 ± 0.04	149 ± 85	
[^{125}I]A-85380	B_{max} (fmol/mg of protein)	16.1 ± 4.8	10.6 ± 2.0	39.6 ± 4.6
	n_H	1.03 ± 0.07	1.10 ± 0.16	1.33 ± 0.32
	K_D (pM)	59.5 ± 28.8	23.7 ± 5.0	56.1 ± 29.8
	K_i for α-CtxMII (nM)	0.11 ± 0.06	98.3 ± 66.1	

TABLE 4

Effect of L-DOPA treatment on high- and low-affinity striatal α -CtxMII-sensitive and α -CtxMII-resistant ^{125}I-epibatidine binding sites

Binding of ^{125}I-epibatidine (400 pM) was inhibited by a range of α -CtxMII concentrations (0.1–3000 nM) using striatal membranes from control and L-DOPA–treated animals. The densities of high- and low-affinity α -CtxMII-sensitive and α -CtxMII-resistant ^{125}I-epibatidine binding sites were determined in each case and compared between control and L-DOPA–treated animals. Each value is the mean ± S.E.M. of four animals.

Treatment Group	α-CtxMII–Sensitive Sites		α-CtxMII–Resistant
	High Affinity	Low Affinity	
	fmol/mg of protein		
Control	9.5 ± 0.6	10.1 ± 1.7	26.2 ± 2.6
L-DOPA	10.5 ± 0.3	4.1 ± 0.7*	25.9 ± 2.1

* $p < 0.02$, significantly different from control animals not receiving L-DOPA.

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ment, although ^{125}I-epibatidine and [^{125}]A-85380 sites were decreased by dopamine precursor administration. To identify the nAChR subtypes(s) involved, we used a filtration membrane-binding assay. This technique has the advantage that it facilitates a thorough characterization of the receptor sites modulated by L-DOPA and makes economical use of the limited monkey tissue available. Analyses were first done using striatal tissue from control monkey brain. Competition studies of ^{125}I-epibatidine and [^{125}]A-85380 with α-CtxMII demonstrated the existence not only of a high-affinity (K_D = 0.15 nM) but also a novel low-affinity (K_i > 100 nM) α-CtxMII-sensitive nAChR. The membrane assay further showed that ^{125}I-epibatidine and [^{125}I]A-85380 bound to similar, if not identical, populations of striatal nAChR populations. Analogous binding studies using striatal tissue from L-DOPA-treated animals show that only the low-affinity α-CtxMII-sensitive population was modulated by dopamine precursor treatment. These results are in agreement with the autoradiographic results, which demonstrate no change in high-affinity α-CtxMII sites after L-DOPA administration.

We next investigated whether L-DOPA treatment modulated nAChRs in MPTP-lesioned animals. The dopamine transporter, measured as an index of nigrostriatal damage, was decreased 80 to 90%. L-DOPA did not affect nAChR binding in MPTP-lesioned animals, despite inducing significant declines in nAChRs in unlesioned animals. These results could be explained in several ways. 1) MPTP treatment may abolish the nAChR subtype(s) regulated by L-DOPA. This would imply that low-affinity α-CtxMII-sensitive nAChRs are found only on dopamine terminals. The residual nAChRs would thus be localized to nondopaminergic striatal elements. Candidates would include striatal GABAergic or cholinergic neurons and incoming glutamatergic or other neurotransmitter afferents from the cortex and other brain regions (Gerfen, 2000; Parent et al., 2000). 2) Alternatively, or in addition, MPTP treatment may destroy sites through which L-DOPA exerts its regulatory effect. For example loss of dopamine terminals, which metabolize L-DOPA to dopamine, might reasonably be expected to reduce L-DOPA's ability to influence striatal neurotransmission. 3) A further possibility is that nAChRs remaining after MPTP treatment are not on the cell surface but intracellular sites subject to unique regulatory influences. This possibility is suggested by our observations that nAChRs are expressed in the internal capsule, a white matter tract extending between the caudate and putamen (Kulak et al., 2002a,b). Studies using subtype selective antibodies are in progress to distinguish between these different alternatives.

The question arose whether L-DOPA treatment also influenced nAChR expression in nondopaminergic regions. The observation that cortical ^{125}I-epibatidine and [^{125}I]A-85380 binding sites were not decreased suggests that the effect of L-DOPA is selective and related to the conversion of L-DOPA to dopamine in dopaminergic nerve terminals. The enhanced striatal dopamine availability would presumably lead to increased dopamine release that could, in turn, trigger a regulatory mechanism that results in nAChR down-regulation.

The observation that primate striatum expresses at least three different nAChR subtypes raises questions as to their subunit composition, an issue that may have important implications for future therapeutic strategies. A converging body of evidence now suggests that high-affinity α-CtxMII-binding sites (those labeled by ^{125}I-α-CtxMII) most likely contain an $\alpha6/\beta2$ interface (Grady et al., 2001; Champtiaux et al., 2002; Whiteaker et al., 2002; Zoli et al., 2002). Evidence also exists for the incorporation of $\alpha4$ and $\beta3$ subunits into at least some of these $\alpha6\beta2$* nAChRs (Zoli et al., 2002; M. Quik, unpublished observation). The involvement of the $\beta3$ subunit is particularly interesting in view of the unusually high $\beta3$ mRNA expression in the substantia nigra (Le Novere et al., 1996; Han et al., 2000; Quik et al., 2000). The composition of the low-affinity α-CtxMII-binding sites remains to be elucidated. The paucity of evidence available at present suggests caution in attempting to attribute a subunit composition to these sites. In contrast, a substantial body of evidence suggests that α-CtxMII-resistant sites in mammalian striatum correspond to $\alpha4\beta2$* nAChRs (Flores et al., 1992; Arroyo-Jimenez et al., 1999; Klink et al., 2001; Jones et al., 2001). Immunochemical investigations by Zoli et al. (2002) suggest that these $\alpha4\beta2$* nAChRs may, in fact, represent $\alpha4\beta2$, $\alpha4\alpha2\beta2$, and/or $\alpha4\alpha5\beta2$ subtypes.

The present results indicate that L-DOPA modulates nAChRs in unlesioned animals, but not in animals with severe MPTP-induced nigrostriatal damage. These two conditions most likely represent extreme ends of the spectrum of Parkinson's disease. If this interpretation is correct, L-DOPA may differentially regulate nAChRs during the course of Parkinson's disease, with a greater influence in the early compared with the later stages of the disorder. Ironically, if low-affinity α-CtxMII-binding sites mediate cholinergic enhancement of dopaminergic transmission, L-DOPA (administered with the objective of enhancing striatal dopamine release) may actually result in a compensatory decline in the cholinergic drive on dopaminergic neurotransmission.

Previous studies using radiolabeled nicotine, methylcarbachol, or epibatidine have shown that there is a loss of nAChRs in the brains of Parkinson's disease patients (Quik and Kulak, 2002). This includes the caudate, putamen, and substantia nigra in which 30 to 75% declines have been observed, as well as the cortex and hippocampus that exhibit decreases of a similar magnitude. These changes in nAChR expression are generally attributed to neurodegenerative processes, but the present results suggest that treatment history may also play a role because the majority of Parkinson's disease patients receive L-DOPA (Ball, 2001; Bezard et al., 2001).

In summary, L-DOPA is very widely used for the treatment of Parkinson's disease. The present results show that L-DOPA administration results in a selective decrease in the expression of low affinity α-CtxMII-sensitive nAChRs in monkey striatum. These L-DOPA-induced declines in nAChRs may be linked to its diminished effectiveness with time and/or the generation of L-DOPA-induced side effects.

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