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Changes of D_1 and D_2 Dopamine Receptor mRNA in the Brains of Monkeys Lesioned with 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine: Correction with Chronic Administration of L-3,4-Dihydroxyphenylalanine

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SUMMARY

The effect of L-3,4-dihydroxyphenylalanine (L-DOPA) on dopamine receptor gene expression in the brain of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys was investigated using *in situ* hybridization histochemistry with measures of changes in relative absorbance. In MPTP-lesioned monkeys, a decrease of D_1 dopamine receptor mRNAs was observed in the rostral part of the caudate and putamen compared with control animals (−20% and −17%, respectively, in the lateral axis). Chronic treatment of MPTP-lesioned monkeys with L-DOPA returned their D_1 receptor mRNA values to near those of control monkeys in the caudate and putamen (92% and 91% of control values, respectively). No lesion or drug-induced changes of D_1 receptor mRNAs were observed in the more caudal parts of the striatum. A decrease of D_1 receptor mRNAs was observed in the olfactory tubercule (−22%) in MPTP-lesioned monkeys compared with control animals but no change was seen in the nucleus accumbens. D_1 receptor mRNAs in the anterior cerebral cortex were decreased in MPTP-lesioned monkeys (−19% compared with control animals). D_1 receptor mRNAs in olfactory tubercle and in cerebral cortex of L-DOPA-treated MPTP-lesioned monkeys were not significantly different from control animals. For D_2 receptor mRNAs, we observed an increase in the caudal part of the caudate and putamen (+24% and +23%, respectively, in MPTP-lesioned monkeys compared with control animals). Chronic L-DOPA treatment corrected this elevation to control values. No variation of D_2 receptor mRNAs was seen in the more rostral parts of the striatum and in the nucleus accumbens in MPTP-lesioned monkeys as well as in MPTP-lesioned monkeys treated chronically with L-DOPA. Our results show for the first time that L-DOPA can influence gene expression of D_1 and D_2 receptors in MPTP-lesioned monkeys and correct the lesion-induced increase in the expression of D_2 receptors, whereas the correction of the D_1 receptor expression decrease is only partial. Furthermore, the changes in gene expression of D_1 and D_2 receptors in MPTP-lesioned monkeys are regional: they are restricted to the anterior striatum for the D_1 receptors and the posterior striatum for the D_2 receptors.

Parkinson's disease is a progressive neurodegenerative disease primarily caused by a loss of dopamine (DA) neurons in the nigrostriatal pathway. Treatment with L-DOPA, the precursor of DA, is still considered the most effective therapy for Parkinson's disease (1). However, long-term treatment with L-DOPA is often associated with the induction of important side-effects such as dyskinesias in humans (2) and monkeys (3–7). It has been postulated that dopaminomimetic-induced dyskinesias is related to the severity of the dopaminergic denervation (8) and to the chronic dopaminomimetic treatment itself (9). However, the neurochemical mechanisms underlying the development of this motor complication still remain unknown.

To understand the action of L-DOPA in Parkinson's disease, it is essential to know the functional state of DA receptors in the basal ganglia in the condition of severe loss of DA neurons. In the brain of Parkinsonians, some studies have demonstrated that both D_1 (10, 11) and D_2 (10, 12) DA receptor densities are increased, whereas others have re-

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ABBREVIATIONS: MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; DA, dopamine; L-DOPA, L-3,4-dihydroxyphenylalanine (levodopa); SSC, NaCl/Na citrate; 6-OHDA, 6-hydroxydopamine; ANOVA, analysis of variance; PLSD, probability of least significant difference.

ported decreased densities of D_2 receptors (13) or no change in either D_1 (14, 15) or D_2 receptors (14, 15). In general, the literature suggests that in untreated Parkinsonism, D_2 receptors densities in the putamen and in the caudate nucleus are either normal or up-regulated (16, 17). In contrast, no general consensus has been established for striatal D_1 receptor densities in either untreated or treated Parkinsonian patients. In the brains of Parkinsonians treated with L-DOPA or with D_2 agonists, D_2 receptors are down-regulated to normal levels (12, 18), whereas D_1 receptors are unaffected by dopaminergic treatment (15). In untreated MPTP-lesioned monkeys, it is generally agreed that D_2 receptors are up-regulated in the striatum (6, 16, 17, 19–21). In contrast, conflicting results have emerged from studies for striatal D_1 receptors; there have been reports both of increase (6, 17, 21) and of no change (16, 22, 23) in receptor density. The increased densities of D_1 and D_2 receptors seen in MPTP-lesioned monkeys were generally reversed after L-DOPA treatment or with selective DA receptor agonists (16, 19, 21, 23).

The present study was designed to evaluate the expression of D_1 and D_2 receptors by measuring their mRNAs through *in situ* hybridization histochemistry in the brain of naive monkeys, untreated MPTP-lesioned monkeys, and in MPTP-lesioned monkeys treated chronically with L-DOPA. The results of these experiments were compared with those we previously obtained (6, 20) in which DA receptor densities were measured by binding techniques using tritiated ligands on brain homogenates and by autoradiography on brain sections. This study investigated the relationship between DA receptor densities and their expression as measured with the mRNAs in the same brain regions.

Materials and Methods

Animals and treatments. Ten drug-naive ovariectomized female cynomolgus monkeys (*macaca fascicularis*; 2.34–3.05 kg) were housed individually and exposed to a 12-hr light/dark cycle. They were fed once per day and had access to water *ad libitum*. Three ovariectomized animals received no treatment and served as normal, healthy controls. Seven monkeys received repeated standard 2-mg subcutaneous doses of MPTP until a satisfactory and stable Parkinsonian syndrome developed. Three of these were treated chronically with L-DOPA (100 mg/kg/day) as well as the peripheral dopa decarboxylase inhibitor Benserazide (25 mg/kg/day) during 1 month.

Tissue preparations. After the end of the chronic dopaminomimetic treatment and after a 3-day drug washout, all monkeys were killed with an overdose of pentobarbital. The brains were removed and placed in isopentane (−40°) and kept frozen at −80°. Hemisected brains were cut into 12-μm coronal sections on a cryostat (−18°); the slices were thaw-mounted onto poly-L-lysine-coated slides (50 μg/ml) and desiccated overnight at 4° before being stored at −80° until use for experiments.

Generation and specificity of cRNA probes. A 734-base pair *Pvu*II restriction fragment (corresponding to base pairs 483 to 1216) and a 362-base pair *Bam*HI-*Pst*I fragment (corresponding to base pairs 653 to 1015) restriction fragment of human D_1 and D_2 receptor cDNA were subcloned into pSP72 (Promega, Madison, WI) and pBluescript SK(+) (Stratagene, La Jolla, CA), respectively (24, 25). Sequence homologies between the human D_1 and D_2 receptor cDNA fragments and the known monkey sequences for these receptors were 98% and 93%, respectively. The sequence selected for D_2 cRNA probe is in a region of the receptor in which complementarity with D3 and D4 receptors is very low. Indeed, when sequences that correspond to the D_2 receptor probe are aligned with the corresponding region of either D_3 or D_4 receptors, a homology of only 32% and 28%, respectively, is obtained, which shows important gaps and mismatches. Such discrepancy is undoubtedly sufficient for the specificity of the probe. Similarly, a 54% homology is obtained when the sequence of the D_1 receptor probe is aligned with the corresponding region of the D_5 receptor gene. At the stringency used in the *in situ* experiments, these probes are highly specific for D_2 and D_1 receptor mRNA. The D_2 receptor probe was designed to identify both of the D_2 receptor splice variants. U(α[^{35}S]thio)TP-labeled antisense- or sense-strand RNA probes were prepared by *in vitro* transcription of linearized templates with the appropriate RNA polymerases using the Riboprobe Gemini System II (Promega).

***In situ* hybridization histochemistry.** The sections were air-dried and stored at room temperature overnight under vacuum with desiccant. The sections were fixed for 20 min in 4% formaldehyde prepared in 0.1 M sodium phosphate buffer (0.08 M Na_2HPO_4, 0.02 M NaH_2PO_4), pH 7.4, at 4° and then rinsed twice for 5 min in 0.1 M potassium phosphate buffer (0.087 M K_2HPO_4, 0.013 M KH_2PO_4, 0.55 M NaCl), pH 7.4, at room temperature. The slides were then immersed in 0.1 proteinase K solution (0.1 μg/ml) for 10 min at 37° and rinsed briefly in diethyl pyrocarbonate-treated water. The slides were incubated in a fresh solution of 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, for 10 min at room temperature. The slide-mounted sections were then rinsed for 5 min in 2× SSC (1× = 150 mM NaCl, 15 mM sodium citrate), dehydrated in a series of ascending concentrations of ethanol, air-dried, and stored at room temperature 1–2 hr under vacuum with desiccant.

For *in situ* hybridization, ^{35}S-labeled RNA probes for D_1 and D_2 receptors were added to their respective hybridization buffers to reach a concentration of 1×10^7 cpm/ml. Hybridization buffer is composed of 50% formamide, 10% dextran sulfate, 0.6 M NaCl, 10 mM Tris, pH 7.5, 1 mM EDTA, pH 8.0, 2.5 × Denhardt's solution, 50 μg/ml yeast tRNA, 15 μg/ml total RNA, 0.1 mg/ml denaturated salmon sperm DNA, and 10 mM dithiothreitol. Ninety microliters of hybridization buffer containing labeled probe were added to each brain section. The sections were then covered with glass coverslips and incubated at 55° for 15 hr. After incubation, the coverslips were floated off in 4× SSC at room temperature and the slide-mounted sections were rinsed briefly in 2× SSC at room temperature. The slides were then washed for 1 hr in 2× SSC at 55°. The slide-mounted sections were treated with RNase A (0.873 units/ml in 10 mM Tris, 0.25 mM EDTA, and 0.5 M NaCl) at 37° for 1 hr and then washed for 1 hr in 2× SSC at 55°. The slides were then washed in a high-stringency solution composed of 0.1× SSC, 14 mM 2-mercaptoethanol, and 0.05% sodium pyrophosphate for 2–3 hr at 60°. The slides were then air-dried and dehydrated in a series of ascending concentrations of ethanol. The slide-mounted tissue sections were then exposed to Kodak BioMax film for 10–14 days at room temperature. The films were developed and the autoradiograms analyzed by densitometry.

mRNAs for D_1 and D_2 receptors were measured at three rostrocaudal coordinates: anterior (A19.0–A22.0); medial (A17.0–A19.0); and posterior (A17.0–A14.0) according to the Szabo and Cowan's Stereotaxis Atlas (26). Caudate and putamen were each divided in two subregions along a medial-lateral axis. mRNAs for DA receptors were measured at the anterior coordinate (A19.0–A22.0) for nucleus accumbens, olfactory tubercle, and cerebral cortex. Data were computed separately for each subregion analyzed. Measures of D_1 and D_2 receptor mRNA were expressed as relative absorbance.

Statistical evaluation. Statistical comparisons of mRNA relative absorbance for D_1 and D_2 receptors were performed by ANOVA followed by pairwise comparisons with Fisher's PLSD test.

Results

Behavioral observations. As previously observed in our laboratory, MPTP-lesioned monkeys were akinetic; L-DOPA treatment produced very good motor recovery but caused

progressive sensitization to treatment and, as expected, induced choreic dyskinesia (data not shown).

Distribution of D_1 and D_2 DA receptor mRNAs. High specific hybridization signal was found in the caudate nucleus and putamen for D_1 and D_2 receptor mRNAs (Figs. 1 and 2). Small but specific D_2 receptor mRNA relative absorbance was detected in the ventral striatum including the nucleus accumbens (data not shown). In contrast, D_1 receptors were highly expressed in the ventral striatum including olfactory tubercle and nucleus accumbens (Fig. 1). In the nucleus accumbens, autoradiograms revealed a heterogeneous signal, with some restricted areas being densely labeled with the D_1 receptor probe. The appearance and location of these densely labeled zones probably represent the islands of Calleja (27). A highly specific hybridization signal was also found in the cerebral cortex for D_1 receptor mRNA (Fig. 1). By contrast, little or no significant expression of D_2 receptors was observed in brain cortical areas (Fig. 2).

Effect of MPTP and L-DOPA on D_1 and D_2 receptor mRNAs. In MPTP-lesioned monkeys, a decrease in relative absorbance for D_1 receptor mRNAs was observed in the rostral part of the caudate and the putamen compared with control animals (−20% and −17%, respectively) (Figs. 1 and 3). No difference was observed between lateral and medial parts of the caudate and putamen at this stereotaxis level. Chronic treatment of MPTP-lesioned monkeys with L-DOPA returned D_1 receptor mRNA relative absorbance near to values for control monkeys in the caudate and putamen (92% and 91% of control values, respectively). In the more caudal parts of the striatum, no change was observed in untreated MPTP-lesioned monkeys and in MPTP-lesioned monkeys treated chronically with L-DOPA compared with controls (Fig. 3).

In the limbic regions of the brain, a decrease of D_1 receptor mRNA relative absorbance was observed in the olfactory tubercule of MPTP-lesioned monkeys compared with control animals (−22%) (Fig. 4). Chronic treatment with L-DOPA slightly increased D_1 mRNA relative absorbance compared with MPTP-lesioned monkeys but this did not reach control value (Fig. 4). In contrast, no change in relative absorbance of D_1 and D_2 receptor mRNAs was seen in the nucleus accumbens of MPTP-lesioned monkeys and those treated chronically with L-DOPA compared with control animals (Fig. 4). D_1 receptor mRNA relative absorbance in the cerebral cortex at the anterior stereotaxic coordinate was decreased in MPTP-lesioned monkeys, whereas in L-DOPA-treated MPTP-lesioned monkeys, absorbance was not significantly different from control animals (Fig. 4).

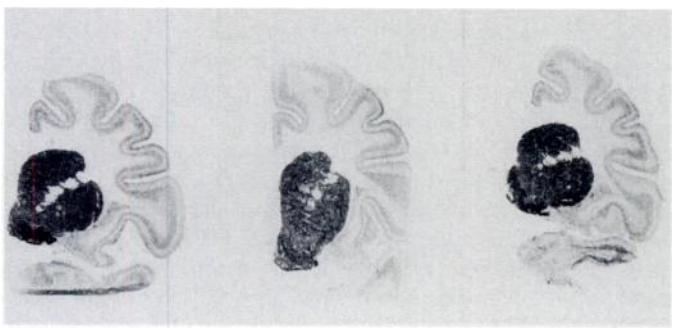

Fig. 1. Photographs of autoradiograms showing D_1 DA receptor mRNA expression at the level of rostral striatum in a control animal, an MPTP-lesioned monkey, and an MPTP-lesioned monkey treated chronically with L-DOPA.

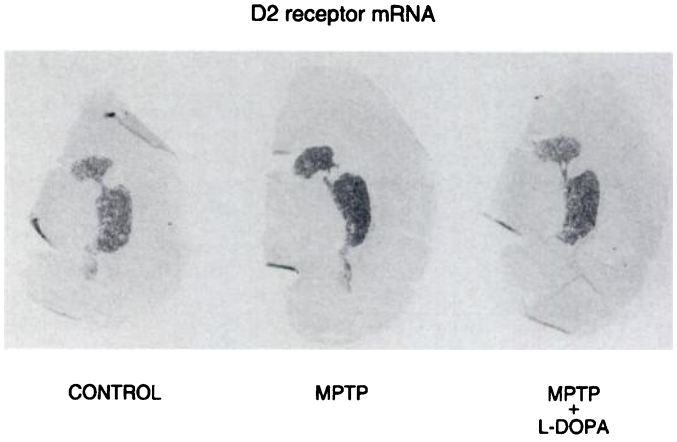

Fig. 2. Photographs of autoradiograms showing D_2 DA receptor mRNA expression at the level of caudal striatum in a control animal, an MPTP-lesioned monkey, and an MPTP-lesioned monkey treated chronically with L-DOPA.

In MPTP-lesioned monkeys, an increase in the relative absorbance of D_2 receptor mRNAs was observed in the caudal part of the caudate and putamen compared with control animals (+24% and +22%, respectively) (Figs. 2 and 3). No difference was observed between lateral and medial parts of the caudate and putamen respectively at this stereotaxis level in MPTP-lesioned monkeys. Relative absorbance of D_2 receptor mRNAs were returned to control values in MPTP-lesioned monkeys treated chronically with L-DOPA (Figs. 2 and 3). No significant change of D_2 receptor mRNA relative absorbance was seen in the more rostral parts of the striatum in MPTP-lesioned monkeys and in MPTP-lesioned monkeys treated chronically with L-DOPA compared with control animals (Figs. 2 and 3). Moreover, no change was observed in relative absorbance of D_2 receptor mRNAs in the nucleus accumbens of MPTP-lesioned monkeys and in MPTP-lesioned monkeys treated chronically with L-DOPA compared with control animals (Fig. 3).

Discussion

In control monkeys, a high specific hybridization signal for D_1 receptors mRNAs was observed in the caudate, putamen, nucleus accumbens, olfactory tubercle, islands of Calleja, and lower levels in the frontal cortex. For D_2 receptors, high specific hybridization signal for D_2 receptors mRNAs were measured in the caudate and putamen with lower levels in the nucleus accumbens. This pattern of distribution of D_1 and D_2 receptor mRNAs is in agreement with other reports that used *in situ* hybridization histochemistry (27, 28) or ribonuclease protection assay analysis (29).

Among monkeys that have been rendered Parkinsonian with the injection of MPTP, this is the first observation of a decrease of D_1 receptor mRNAs in the rostral part of the caudate nucleus and the putamen; an increase in D_2 receptor mRNAs was observed in the more caudal parts of the brain in

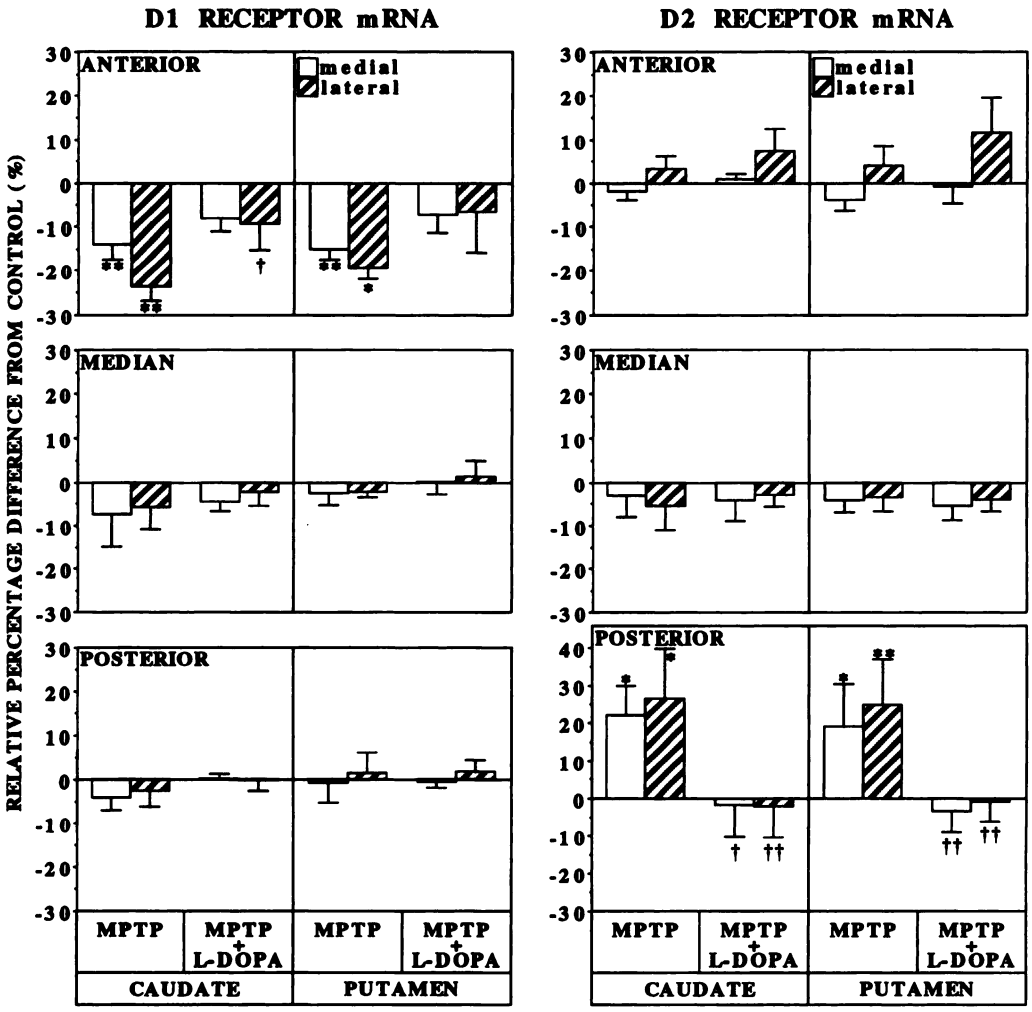

Fig. 3. Effect of chronic administration of L-DOPA on striatal D_1 and D_2 DA receptor mRNA expression in MPTP-lesioned *m. fascicularis* monkeys at three rostro-caudal coordinates. Results are expressed as a percentage difference (mean ± standard error) of mean mRNA relative absorbance measured in control animals. The data were obtained from three controls, four MPTP-lesioned monkeys, and three MPTP-lesioned monkeys treated chronically with L-DOPA. *, $p < 0.05$; **, $p < 0.01$ compared with respective control animals; †, $p < 0.05$; ††, $p < 0.01$ compared with respective MPTP-lesioned animals determined with a one-factor ANOVA followed by pairwise comparisons with the Fisher's PLSD test.

the same structures. This is in agreement with our previous D_2 receptor studies in caudate-putamen of MPTP-lesioned monkeys with saturation binding experiments using the antagonist ligand [^{3}H]spiperone or the agonist [^{3}H]*N*-propylnorapomorphine in tissue homogenates as well as by autoradiography that showed increased D_2 receptor density (6, 19, 21, 30). The changes observed here in relative absorbance of D_2 receptor mRNAs concentrated in the posterior striatum is also in agreement with our recent findings of elevated [^{3}H]spiperone and [^{3}H]*N*-propylnorapomorphine in the posterior but not the anterior caudate-putamen of MPTP-lesioned monkeys (30).

Accordingly, several studies have demonstrated such a relationship for striatal D_2 receptors in rat after lesion of midbrain DA neurons with 6-hydroxydopamine (6-OHDA), a treatment known to produce an up-regulation of striatal D_2 receptors (31–34). Furthermore, it is well known that prolonged administration of neuroleptics to rats increases the density of D_2 receptors in the striatum (35). Thus, numerous studies have shown that increased rat striatal D_2 receptors density seen after chronic treatment with neuroleptics is associated with an increase in the content of its corresponding mRNA (32, 34, 36–39). Those results and the results obtained in the present study suggest that prolonged DA denervation or D_2 receptor blockade is regulated to some extent at the level of gene expression by increasing gene transcription and/or mRNA stability. It is likely that dopaminergic neurotransmission exerts tonic inhibition on D_2 mRNA and receptor levels in the caudal parts of caudate nucleus and putamen in monkeys. Thus, we have shown that chronic treatment of MPTP-lesioned monkeys with L-DOPA returned D_2 receptor mRNA levels to that of naive control monkeys. Furthermore, it has been demonstrated that chronic L-DOPA treatment reverses the increased densities of striatal D_2 receptors in MPTP-lesioned monkeys (16, 21, 40). Similar observations have been made in 6-OHDA rats treated chronically with the D_2 agonist quinpirole or with DA (41, 42). However, in a recent study in monkeys, MPTP led to a small increase of putaminal D_2 receptor density that was not corrected by chronic L-DOPA treatment (6, 20).

In MPTP-lesioned monkeys, slight increases (6, 17, 21) or no change (16, 22, 23) in striatal D_1 receptors densities have been observed. Furthermore, studies of MPTP-lesioned monkeys have been reported in which D_1 receptors are up-regulated after overstimulation by chronic treatment with L-DOPA (6, 17). Surprisingly, we observed a decrease in relative absorbance for D_1 receptor mRNAs in the anterior caudate nucleus and putamen of MPTP-lesioned monkeys compared with controls. By contrast, relative absorbance of D_1 receptor mRNAs in the caudate nucleus and putamen of L-DOPA-treated MPTP-lesioned monkeys was not different from controls and significantly lower compared with MPTP-

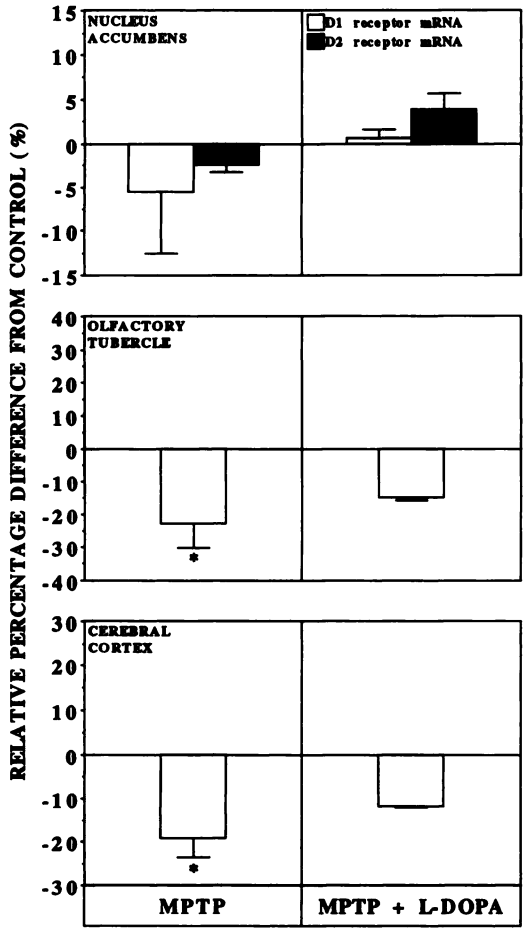

Fig. 4. Effect of chronic administration of levodopa (L-DOPA) in MPTP-lesioned *m. fascicularis* monkeys on D_1 and D_2 DA receptor mRNA expression in nucleus accumbens and on D_1 DA receptor mRNA expression in olfactory tubercle and cerebral cortex. Results are expressed as a percentage difference (mean ± standard error) of mean mRNA relative absorbance measured in control animals. The data were obtained from three controls, four MPTP-lesioned monkeys, and three MPTP-lesioned monkeys treated chronically with L-DOPA. *, $p < 0.05$ compared with respective control animals determined with a one-factor ANOVA followed by pairwise comparisons with the Fisher's PLSD test.

lesioned monkeys in the anterolateral caudate, which suggests a correction by L-DOPA therapy. A decrease of striatal D_1 receptor mRNAs has also been observed in the striatum of 6-OHDA-lesioned mouse and rat (36, 42). It is likely that by opposition to D_2 receptors, dopaminergic neurotransmission exerts a tonic stimulation on D_1 mRNA and receptor levels in the striatum. Interestingly, lesion- and treatment-induced changes of D_1 receptor mRNAs in the striatum of 6-OHDA rats and MPTP-lesioned monkeys parallel the changes of the density of the D_1 agonist site (30, 43). Hence, denervation induces a decrease of D_1 high affinity agonist sites and D_1 receptor mRNAs and this is corrected with agonist treatment (30, 43). Furthermore, in MPTP-lesioned monkeys, bromocriptine corrected significantly the [^{3}H]SKF 38393 binding to D_1 receptors in the anterior striatum (30). Therefore, the density of the high affinity D_1 receptor agonist sites correlates better with the mRNA for D_1 receptors than with the antagonist site. For D_1 receptors, we observed in MPTP-lesioned monkeys that the response of antagonist and agonist sites of D_1 receptors to denervation is diametrically opposed, whereas for D_2 receptors a similar response is observed (30). MPTP treatment increased D_2 receptor mRNA in the caudal striatum, a region that corresponds mainly to sensorimotor territory (44). In contrast, MPTP treatment decreased D_1 receptor mRNA in the rostral striatum, a region that corresponds mainly to associative territory (44). It is unlikely that these regional changes are caused by uneven dopaminergic denervation because DA content was uniformly reduced in the striatum of these MPTP-lesioned monkeys.[1] The effect of MPTP on striatal enkephalin and substance P expression shows no marked rostro-caudal differences (45–47), which is in contrast to the regional D_1 and D_2 receptor mRNA changes observed here. The response of substance P mRNA to L-DOPA was more pronounced in the caudal striatum and in lateral subregions of the rostral caudate nucleus of MPTP marmosets (45). Our findings support the idea of an up-regulation of D_2 gene expression in Parkinsonian monkeys and provide evidence that this phenomenon is involved in the processing of sensorimotor information. The D_1 receptor mRNA decrease observed in striatal and cortical areas after MPTP DA depletion, which is similar to that of Parkinson's disease, may be detrimental to normal cognitive processing (48).

The molecular mechanisms responsible for the differential regulation of D_1 and D_2 receptors by dopaminergic neurotransmission is complex and probably involves various cellular substances induced by the stimulation of DA receptors acting in concert to control DA gene expression. Indeed, DA differentially regulates the two major output pathways from the striatum; the striatopallidal projection (indirect pathway), which coexpresses glutamic acid decarboxylase, and enkephalin and is predominantly regulated by D_2 receptors, and the striatonigral projection (direct pathway), which is regulated by D_1 receptors and coexpresses glutamic acid decarboxylase, dynorphin, and the tachykinin substance P (49). In 6-OHDA rats, several studies have shown that the levels of enkephalin mRNA are increased in the striatopallidal pathway, whereas the levels of substance P are decreased in the striatonigral pathway (42, 45). These results suggest that the D_2 receptors exert a tonic inhibitory regulation of enkephalin expression in the striatopallidal neurons, whereas D_1 receptors exert a tonic stimulatory regulation of substance P expression in the striatonigral neurons. Indeed, in 6-OHDA rats, treatment with selective D_1 and D_2 receptor agonists reverses the decrease of substance P mRNA levels and the increase in the levels of enkephalin mRNA, respectively (42, 50). In contrast, L-DOPA replacement therapy produces a complete reversal of the lesion-induced decrease in substance P mRNA but fails to reverse the increase in enkephalin mRNA in 6-OHDA rats (51) and in MPTP-lesioned monkeys (45, 46). It seems that chronic L-DOPA treatment produces a new functional state in the striatum, as proposed by Engber *et al.* (51). L-DOPA probably induces a shift from one imbalance to a new imbalance between the direct and indirect pathway activities. In MPTP-lesioned primates, a decrease in substance P mRNAs and an increase in preproenkephalin mRNAs are already apparent 7 days after MPTP treatment (45), a time in which no supersensitive DA receptors would be installed. Changes in D_2 receptor densities and their corresponding mRNA could be a compensatory mechanism to restore altered neuropeptide expression in the

[1] T. Di Paolo, unpublished observations.

striatopallidal output neurons after destruction of ascending dopaminergic projections. Increased density of the D_2 receptor subtype in the indirect pathway could accentuate the tonic inhibitory activity to correct preproenkephalin gene expression. However, our results showed that treatment of MPTP-lesioned monkeys with L-DOPA restored D_2 mRNA levels; previous reports, however, find that this treatment fails to reverse the increase in preproenkephalin mRNA (45, 46). These results suggest that the functional interaction between D_2 receptor and enkephalin in the indirect pathway is disrupted after MPTP treatment and is not restored by L-DOPA treatment. The mechanisms underlying this effect are unknown. It is also possible that the response of enkephalin to L-DOPA is the resultant of DA acting not only on D_2 receptors but also on the other DA receptor subtypes that may be opposing the D_2 receptor response. The possible compensatory mechanism after DA depletion by MPTP described above for D_2 receptors seems unlikely for the D_1 receptor subtype given the results of the present study. Indeed, we observed a decrease of D_1 receptor mRNA after MPTP treatment and an increase with L-DOPA therapy; a similar pattern is observed for changes in substance P (45, 46). An increased tonic stimulatory regulation by the D_1 receptor subtype would be expected to restore substance P expression. Nevertheless, these results suggest that the functional interaction between D_1 receptors and the neuropeptide substance P in the striatonigral neurons is preserved after DA denervation and that the underactivity of these neurons can be corrected by a chronic activation of a D_1 receptor subtype. The results are consistent with observations that show relative normal activity of the D_1 direct pathway after L-DOPA treatment (52, 53). The abnormal functional state of the indirect pathway and the normal functional state of direct pathway may be related to the onset of dyskinesia seen after chronic L-DOPA therapy. Neurotransmitters and receptors other than DA D_1 and D_2 receptors may be implicated in the striatal peptide regulation because the DA receptor changes are restricted regionally, whereas for peptides, it is a more general effect. Anatomical differences and neurotransmitter interactions may contribute to the differential regulation of D_1 and D_2 receptors. The regulation of striatal D_1 and D_2 receptors mRNA levels in MPTP-lesioned monkeys and after chronic treatment with L-DOPA could constitute an important clue for elucidating the mechanisms underlying induction of dyskinesia. Indeed, the regulation of DA receptor expression after L-DOPA treatment observed in this study and the effect of this treatment on other cellular components of the output pathways from the striatum seen in other studies, particularly neuropeptides, may provide insight into the pathophysiology of dyskinesia induced by long term L-DOPA therapy in Parkinsonian patients.

In summary, this is the first study in monkeys to report that MPTP denervation causes an imbalance of brain DA receptor expression with a decrease of D_1 receptor mRNA in the rostral part of the caudate-putamen and an increase for the D_2 receptor mRNA in the more caudal region of the caudate-putamen. This imbalance is corrected with chronic L-DOPA treatment for D_2 receptors. Expression of D_1 receptors in the anterolateral caudate of L-DOPA-treated MPTP-lesioned monkeys is not different from controls and is significantly lower than in MPTP-lesioned monkeys, which suggests a partial correction with L-DOPA therapy.

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