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ORIGINAL ARTICLE

Metabotropic Glutamate Receptor II in the Brains of Parkinsonian Patients

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Abstract

Modulation of basal ganglia group II metabotropic glutamate receptors (mGluR2/3) is a potential therapeutic alternative to levodopa in Parkinson disease (PD). We used receptor-binding autoradiography of the mGluR2/3-selective radioligand [^{3}H]LY341495 in postmortem brain specimens from PD patients (n = 14) and controls (n = 11) to investigate possible contributions of changes in ligand binding of this receptor to levodopa-associated motor complications experienced premortem in PD patients. The PD patients included those with and without histories of dyskinesias and those with and without ''wearing off,'' which is defined as a reduced period of benefit from levodopa. Specific binding of [^{3}H]LY341495 to mGluR2/3 in the basal ganglia was higher in the caudate nucleus than the putamen and lower by approximately half in the external and internal globus pallidus (GPi) in controls. [^{3}H]LY341495-specific binding was reduced in the caudate and GPi in patients without wearing-off (−22% caudate, −30% GPi), compared with controls and with patients who had experienced wearing-off; there were no differences among PD patients with or without dyskinesias. These data suggest that an adaptive downregulation of mGluR2/3 in PD patients without wearing-off may compensate for increased glutamate. They indicate a key role for mGluR2/3 in control of movement and the potential for mGluR2/3-targeted drugs in the management of wearing-off fluctuations in PD.

Key Words: Dyskinesias, mGluR2/3, Parkinson disease, Wearing-off.

INTRODUCTION

Parkinson disease (PD) is a progressive neurodegenerative disorder characterized by tremor, rigidity, bradykinesia, and instability in postural reflexes that is primarily attributed to loss of dopamine neurons in the substantia nigra compacta (1). Although levodopa remains the gold standard for symptomatic treatment of PD (2), various complications including motor fluctuations such as ''wearing-off'' and ''on-off'' phenomena and abnormal involuntary movements (i.e. levodopa-induced dyskinesias [LIDs]) limit the quality of life in PD patients. Recent studies have reported that 38% to 50% of patients develop motor complications within 2 years, and 50% to 80% of patients develop motor complications within 5 to 10 years of therapy (3, 4). ''Wearing-off'' is defined as a reduced duration of benefit from an individual levodopa dose and a recurrence of parkinsonian symptoms before the normal dosing schedule of levodopa and is the first type of motor fluctuation to develop within 5 to 6 months of therapy (5).

A large body of evidence suggests that increased glutamatergic transmission in the basal ganglia motor circuit plays a critical role in the clinical manifestations of PD and in levodopa-induced motor complications (6–8). In addition to the fast synaptic transmission mediated by ionotropic glutamate receptors, metabotropic glutamate receptors (mGluRs) are of particular interest because of their abundance in the basal ganglia and their ability to respond to low concentrations of glutamate to modulate neuronal excitability (9, 10). The mGluRs constitute a family of 8 receptor subtypes that are classified into 3 groups based on the homology of the amino acid sequence, signal transduction pathway, and receptor pharmacology (11). Group I receptors (mGluR1 and mGluR5) are coupled to activation of phospholipase C and activate protein kinase C. Group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7, and mGluR8) receptors are negatively coupled to adenylyl cyclase and inhibit adenosine 3*c*,5*c*-cyclic monophosphate (cAMP) formation. Group II mGluRs are also linked to the rapid regulation of various voltage-dependent Ca^{+2} and K^{+} channels (11). In the striatum, mGluRII are located presynaptically on excitatory corticostriatal terminals, cholinergic interneuron terminals, and GABAergic medium spiny output neurons in rat, primate, and human striatum (10, 12–16). Group II mGluRs are also localized in the external and internal segments of globus pallidus (GPe and GPi, respectively) and presynaptically on subthalamic nucleus (STN) terminals in the substantia nigra reticulata (SNr) (10, 14, 15, 17). Whereas mGluR3 is widely distributed in neuronal and glial cells, mGluR2 is only expressed in neurons (11, 18, 19). Large differences in mRNA expression between mGluR2 and mGluR3 have been identified in rat brain (12).

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There are close interactions between mGluR2/3 and dopaminergic systems both presynaptically and postsynaptically (20, 21), but the precise role of mGluR2/3 in basal ganglia functions is not well known; available data are inconclusive regarding the effect of selective activation or blockade of mGluR2/3 in the regulation of movement in animal models of PD. In rats, selective mGluR2/3 agonists produced antiparkinsonian-like effects in reserpine-induced akinesia (22) and haloperidol-induced catalepsy (17), but intrastriatal administration of an mGluR2/3 agonist did not reduce parkinsonian-like symptoms (23). Group II mGluR antagonists also increase basal locomotor activity (21), and additive effects of an mGluR2/3 antagonist with the dopamine agonist apomorphine and with L-Dopa have also been reported in reserpine and unilateral 6-hydroxydopamine–lesioned rat models (24). Results from unilateral 6-hydroxydopamine–lesioned rat models of PD are varied with increased (25) or no change (16) in striatal mGluR2/3 expression and even decreased striatal and pallidal mGluR3 mRNA expression (26). In monkeys, specific binding to mGluR2/3 receptors in the basal ganglia remained unchanged by the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesion and L-Dopa treatment inducing dyskinesias (27).

This study was undertaken to determine whether adaptive changes in the basal ganglia mGluRII are associated with the development of motor complications in levodopa-treated parkinsonian patients. We report that patients with histories of the absence of wearing-off phenomenon but not LIDs have a reduction of [^{3}H]LY341495-specific binding to mGluR2/3 in the caudate nucleus and GPi compared with controls and with levodopa-treated PD patients with histories of wearing-off.

MATERIALS AND METHODS

Patients

The patients studied were the same as those previously investigated (6, 28–30). These patients were selected from a large prospective study on levodopa-induced motor complications, evaluated by the same neurologist (A.H.R.) at 6- to 12-month intervals (31). The clinical profiles of the PD patients, the age and mode of onset, severity of the disease, drug treatment, response to treatment, and motor complications (LIDs, wearing-off) were entered prospectively after each clinical assessment (31). All motor complications after each levodopa dose are defined based on information reported by the family, another observer, or the family physician and confirmed by the neurologist during a personal interview with the patient or family, as well as evidence of these motor abnormalities at the time of neurological examination. Levodopa-induced dyskinesias are defined as biphasic or peak-dose choreic/dystonic abnormal involuntary movements; ''wearing-off'' is defined as a predictable decline in motor benefit at the end of the dose in a patient with previously stable response (31). The patients were analyzed in relation to the presence (n = 7) or absence (n = 7) of dyskinesias, and the presence (n = 6) or absence (n = 8) of wearing-off (Table 1). These groups were not statistically different with respect to sex, age of death, brain pH, age of PD onset, duration of PD, duration of levodopa use, average

TABLE 1. Biochemical and Clinical Data of Parkinson Disease Patients

	Dyskinesia		Wearing-off	
Data*	**Nondyskinetic**	**Dyskinetic**	**No Wearing-off**	**Wearing-off**
N	7 (1W, 6M)	7 (3W, 4M)	8 (3W, 5M)	6 (1W, 5M)
Delay to autopsy, hour	14 ± 2†	11 ± 3†	13 ± 3†	12 ± 3†
Age of death, year	80 ± 3‡	77 ± 2‡	78 ± 2‡	78 ± 3‡
Brain pH	6.36 ± 0.05§	6.39 ± 0.04§	6.22 ± 0.04§	6.44 ± 0.02§
Age of PD onset, year	63 ± 3	61 ± 2	64 ± 3	60 ± 2
Duration of PD, year	17.4 ± 3.5	15.4 ± 2.4	14.6 ± 2.4	18.5 ± 3.6
Duration of LD use, year	11.9 ± 2.8	10.7 ± 1.8	11.6 ± 2.2	10.9 ± 2.5
Cumulative LD use, g	15,316 ± 6,422	11,984 ± 2,884	11,936 ± 3,765	15,937 ± 6,511
Duration of follow-up, year	11.6 ± 1.9	10.4 ± 1.7	10.7 ± 1.6	11.3 ± 2.2
Age at LD initiation, year	68 ± 3	64 ± 2	66 ± 3	66 ± 2
Duration of PD at LD initiation, year	5.3 ± 1.5	3.1 ± 1.1	3.0 ± 0.9	5.8 ± 1.7
Daily LD dose, g	2.8 ± 0.7	3.7 ± 0.9	2.4 ± 0.4	4.3 ± 1.0
Anti-parkinsonian medication (N)				
Levodopa	7	7	8	6
Levodopa-controlled release	2	5	3	4
Amantadine	5	4	5	4
Dopamine agonist	4	4	5	3
Selegiline	3	3	4	2
Anticholinergic	6	6	6	6

*Eleven control subjects were also studied (see Materials and Methods section).
†Delay to autopsy of the control subjects was less than 24 hours and was not significantly different from the delays for all the subgroups of PD patients.
‡$p < 0.01$ vs ages of death of control subjects.
§Not significantly different from brain pH of control subjects which was 6.40 ± 0.07.
PD, Parkinson disease; LD, levodopa; N, number of patients; W, women; M, men.

daily dose of levodopa, cumulative levodopa dose, duration of follow-up, and age at levodopa initiation (table and previous publications [6, 28–30]). The patient medical records were rechecked to determine the time of the last levodopa and/or dopamine agonist treatment. Two patients had received levodopa shortly (3 hours) before death but not the others; for 1 patient, this information was not available.

Eleven control patients (2 women and 9 men) were also studied. Delay to autopsy of the control subjects was less than 24 hours and was not significantly different from the delays for the PD patients (Table 1). The age of death of control subjects was 68 ± 3 years ($p < 0.01$ vs age of death of PD patients).

Autopsy and Handling of Brain Material

All autopsies of the 14 PD patients and the 11 control patients (2 from Douglas Hospital Research Center brain bank, Montreal, Canada) who died with no neurological disorders were performed within 24 hours of death. The brains were immediately divided into halves; one half was frozen at −80°C for biochemical studies and the other half was fixed in formalin for histological studies. These half brains were chosen at random for biochemical or histological studies. The PD diagnosis was based on marked neuronal loss in the substantia nigra compacta, the presence of Lewy body inclusions, and the absence of other pathological changes that might have accounted for parkinsonian symptoms (31). The frozen half brain was cut by hand in the frontal plane into 2- to 3-mm-thick slices.

Tissue Preparation

Small punches of the cerebral cortex were used to determine tissue pH as an assessment of its preservation (32). Brain slices were cut into coronal sections (20 μm) on a cryostat (−18°C), thaw-mounted onto Superfrost plus 75 × 50-mm slides (Fisher; Nepean, Ontario, Canada), desiccated overnight at 4°C, and stored at −80°C until they were assayed. Small extracts of putamen were dissected, stored at −80°C, and processed for measurements of biogenic amine concentrations.

Measures of the Extent of Dopamine Denervation

The extent of striatal dopamine denervation was assessed by measurement of dopamine concentrations and dopamine transporter–specific binding. Biogenic amine concentrations were assayed by high-performance liquid chromatography with electrochemical detection, and the dopamine transporter was labeled with [^{125}I]RTI-121 (3β*a*-(4-^{125}I-iodophenyl) tropane-2β-carboxylic acid isopropyl ester), as previously reported (29).

[^{3}H]LY341495 Autoradiography

Group II mGluRs receptor-specific binding was evaluated using [^{3}H]LY341495 (34.61 Ci/mmol) (Tocris Cookson, Ltd, Bristol, UK) binding by an adaptation of previously published methods used on rat brain (33). Sections were preincubated for 30 minutes in ice-cold phosphate-bromide buffer (10 mM potassium phosphate buffer with 100 mM potassium bromide), pH 7.6. After drying, the sections were incubated for 90 minutes at room temperature with 5 nM [^{3}H]LY341495 in phosphate-bromide buffer. Nonspecific binding was determined in the presence of 1 mM L-glutamic acid (Research Biomedical International, Natick, MD) in the buffer solution. After 2 washes for 30 seconds in ice-cold phosphate-bromide buffer, the sections were rinsed briefly (30 seconds) in ice-cold distilled water. Finally, the slide-mounted tissue sections were dried overnight at room temperature and exposed to ^{3}H-sensitive films (Kodak, Rochester, NY; along with standards [^{3}H]-microscales; Amersham, Buckinghamshire, UK) for 14 days at room temperature.

Data Analysis and Statistics

Intensities of autoradiographic labeling with [^{3}H]LY341495 were quantified on x-ray films by a Power Macintosh G4 connected to a video camera (XC-77; Sony, Toronto, Canada) and a constant illumination light table using computerized densitometry (National Institutes of Health image v.1.63). Optical gray densities were transformed into nanocuries per milligram of tissue equivalent using a standard curve generated with [^{3}H]-standards. The results were then converted into femtomoles per milligram of tissue using the specific activity of the radioligand. The caudate nucleus and the putamen were quantified in 4 subregions along a medial-lateral and dorsoventral axis. The GPe and GPi were divided along a dorsoventral axis. Nonspecific binding was measured in the same brain regions on adjacent sections and then subtracted from total binding. Control subjects (n = 11) were used for the distribution analyses of mGluR2/3 in the basal ganglia and were compared with paired *t*-tests. Statistical comparisons of all other data were performed by analysis of variance, followed by post hoc pairwise comparisons with Fisher probability of least significant difference test. All control (n = 11) and PD patients (n = 14) were compared first. Comparisons were then performed between controls, PD patients who had developed LIDs, and PD patients who had not developed LIDs. Finally, comparisons were made among controls, PD patients with wearing-off, and PD patients without wearing-off. A value of $p = 0.05$ or less was considered significant.

RESULTS

Biogenic amine concentrations measured by high-performance liquid chromatography in the same brain specimens were previously reported (29). There was a marked decrease of dopamine (−98.8%), homovanillic acid (HVA; −78.3%), 3-methoxytyramine (−91.4%), and to a lesser extent 5-hydroxytyramine (−48.5%) and 5-hydroxy-indolacetic acid (−44.8%) concentrations in the putamen of all PD patients compared with controls; there were no difference among the subgroups of PD patients. The loss of striatal dopaminergic innervation was also evaluated previously with [^{125}I]RTI-121–specific binding to the dopamine transporter (29). There was a marked decrease of dopamine transporter in the caudate nucleus (−72%) and putamen (−92%) of all PD patients compared with controls, without difference among PD patient sub-groups.

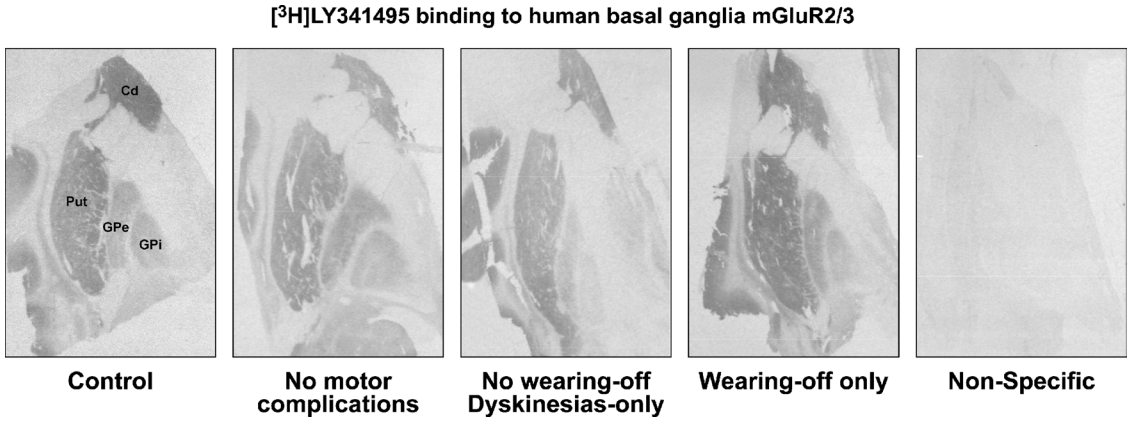

FIGURE 1. Representative autoradiograms of [^{3}H]LY341495 binding in postmortem human coronal brain sections at the 14.6-mm level (34) showing a control subject and levodopa-treated parkinsonian patients who had or had not developed motor complications; an example of nonspecific binding in the presence of 1 mM L-glutamic acid is also shown. Cd, caudate nucleus; Put, putamen; GPe, external globus pallidus; GPi, internal globus pallidus.

Representative autoradiograms of [^{3}H]LY341495 binding in controls and different groups of PD patients and nonspecific binding are shown in Figure 1. There was higher [^{3}H]LY341495-specific binding in the caudate nucleus (30%) of control subjects than in the putamen (df = 10, t = 8.1, $p < 0.0001$); this was approximately twice the binding measured in the globus pallidus (caudate vs GPe: df = 10, t = 20.1, $p < 0.0001$; caudate vs GPi: df = 10, t = 23.1, $p < 0.0001$; putamen vs GPe: df = 10, t =11.9, $p < 0.0001$); putamen vs GPi: df = 10, t =13.0, $p < 0.0001$) (Figs. 1, 2). There was a small (12%) but significantly higher [^{3}H]LY341495-specific binding in the GPe compared with the GPi (df = 10, t = 2.9, $p = 0.0164$). Division of these brain areas in their dorsal, ventral, lateral, and medial subregions showed smaller differences (10% or less).

Lesion and treatment effects were similar in all subregions of the caudate nucleus, putamen, and GPe and GPi; therefore, the values of the subregions were grouped in the subsequent analyses. [^{3}H]LY341495-specific binding of the 2 patients who had received levodopa shortly before death was not different from that of the other PD patients.The loss of dopaminergic innervation was associated with a significant reduction of [^{3}H]LY341495-specific binding in the caudate nucleus of all PD patients (−13%) compared with controls ($F_{1,23}$ =5.01, $p = 0.04$; Fig. 3). When PD patients were analyzed according to the presence or absence of dyskinesias, no significant effect on [^{3}H]LY341495-specific binding was observed in the caudate nucleus ($F_{2,22}$ = 2.44, $p = 0.11$; Fig. 3). Binding was, however, reduced significantly in the caudate of PD patients who had not developed wearing-off complications (−20% to −22%) compared with patients with wearing-off ($p < 0.01$) and controls ($F_{2,22}$ = 6.93, $p = 0.005$), respectively (Fig. 3).

[^{3}H]LY341495-specific binding was not different in the putamen of all PD patients compared with controls ($F_{1,23}$ = 0.28, $p = 0.60$; Fig. 3). Moreover, this binding was not altered in the putamen when patients with PD were grouped according to the development or absence of dyskinesias or wearing-off ($F_{2,22}$ = 1.18, $p = 0.33$; $F_{2,22}$ = 1.75, $p = 0.20$, respectively) (Fig. 3).

[^{3}H]LY341495-specific binding was not changed in the GPe of all PD patients ($F_{1,23}$ = 1.90, $p = 0.18$) or different groups of patients who had or had not developed dyskinesias or wearing-off ($F_{2,22}$ = 1.01, $p = 0.38$; $F_{2,22}$ =1.17, $p = 0.33$, respectively) (Fig. 4). By contrast, binding was reduced significantly (−25%) in the GPi of all PD patients compared with controls ($F_{1,23}$ = 5.86, $p = 0.02$; Fig. 4). This reduction did not reach significance in the GPi of patients with or without dyskinesias ($F_{2,22}$ = 2.82, $p = 0.08$), but it was less in patients who had not developed wearing-off (−30%)

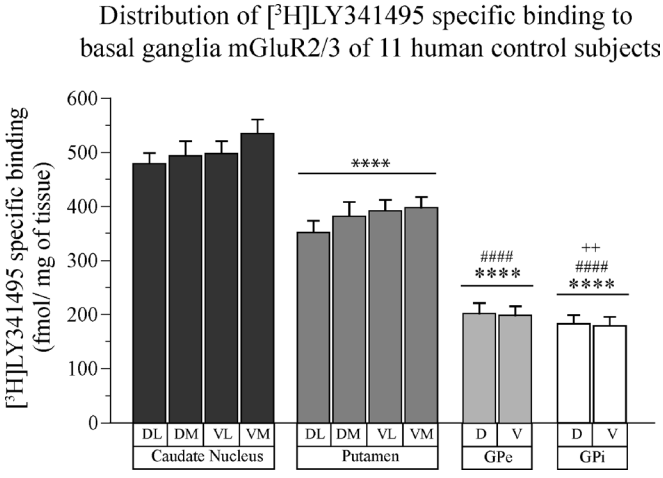

FIGURE 2. [^{3}H]LY341495 binding in the caudate nucleus, putamen, and globus pallidus (external [GPe] and internal [GPi]) of postmortem human controls. Values are presented in femtomoles per milligram (fmol/mg) of tissue as mean ± SEM. Higher [^{3}H]LY341495-specific binding was observed in the caudate nucleus compared with the putamen, and both of these bindings were twice those measured in the GPe or GPi. In the globus pallidus, [^{3}H]LY341495-specific binding was higher in the GPe compared with the GPi. ****$p < 0.0001$ vs caudate nucleus; $^{\#\#\#\#}p < 0.0001$ vs putamen; $^{++}p = 0.0164$ vs GPe. DL, dorsolateral; DM, dorsomedial; VL, ventrolateral; VM, ventromedial; D, dorsal; V, ventral.

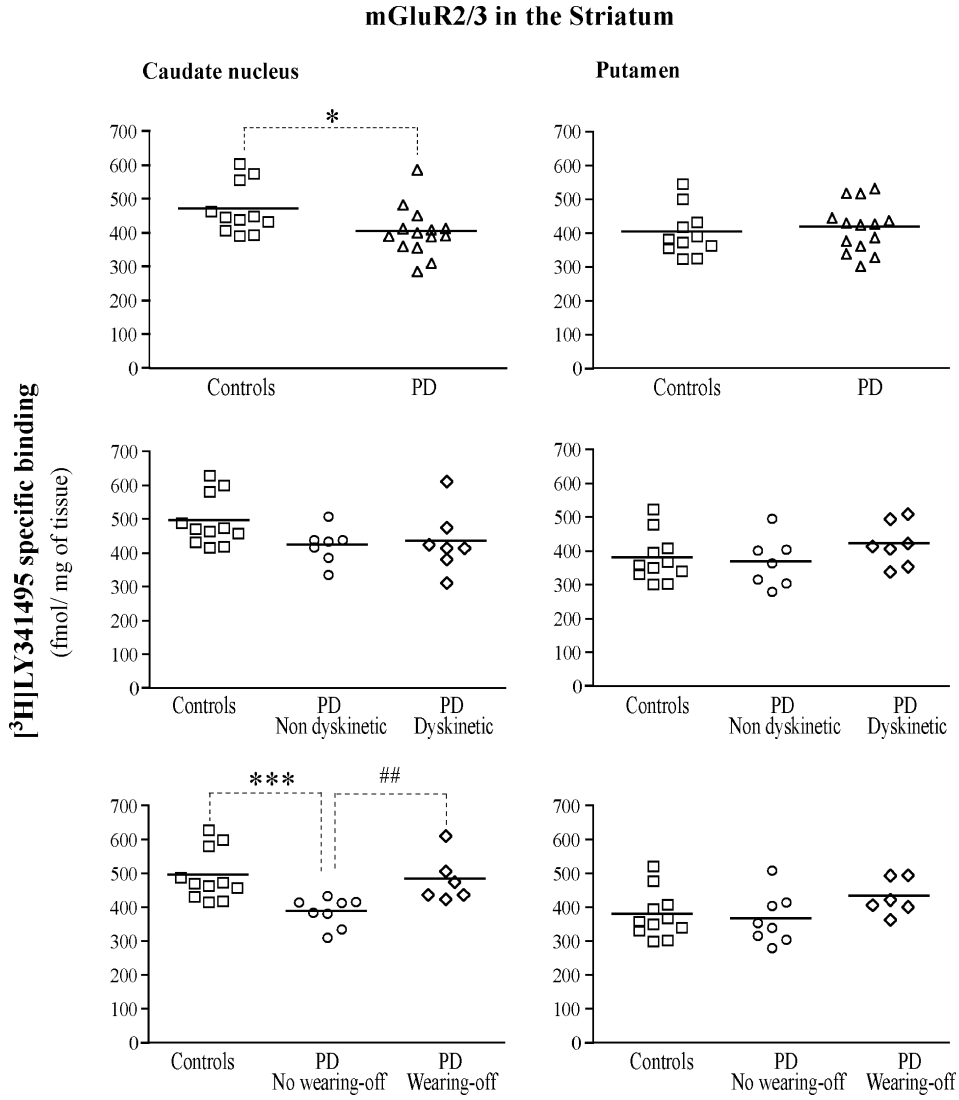

FIGURE 3. [^{3}H]LY341495 binding in the caudate nucleus and putamen of postmortem control and levodopa-treated parkinsonian patients. Individual values and means are in femtomoles per milligram (fmol/mg) of tissue. Significantly less [^{3}H]LY341495 binding was observed in the caudate nucleus but not putamen of Parkinson disease (PD) patients compared with controls (−13%). No differences in [^{3}H]LY341495-specific binding were observed in the caudate and putamen of PD patients who had or had not developed dyskinesias. There was less binding in the caudate nucleus of PD patients who had not developed wearing-off motor complications compared with controls (−22%) and to PD patients who had wearing-off fluctuations (−20%). *$p < 0.05$ and ***$p < 0.005$ vs controls; $^{\#\#}p < 0.01$ vs PD wearing-off.

fluctuations compared with control subjects ($F_{2,22} = 3.34$, $p = 0.05$; Fig. 4).

DISCUSSION

The present study is the first to show the following: 1) [^{3}H]LY341495-specific binding to mGluR2/3 in normal controls is higher in the caudate nucleus than in the putamen and that both are approximately twice as high as in the globus pallidus, with the former more in the external than internal part; 2) there is lower [^{3}H]LY341495-specific binding in the caudate nucleus and GPi of patients who had not had wearing-off compared with controls and to PD patients who had developed wearing-off; and 3) there is similar [^{3}H]LY341495-specific binding in the caudate nucleus, putamen, and GPe and GPi of PD patients who did or did not have dyskinesias.

The distribution of [^{3}H]LY341495-specific binding observed in human control subjects is similar to that observed in monkeys (27). As in MPTP-treated monkeys (27), [^{3}H]LY341495 specific binding was not different in dyskinetic and nondyskinetic subjects and controls. The MPTP monkey experiments were not designed to study wearing-off, and it was not observed in these monkeys. There was, however a decrease of [^{3}H]LY341495-specific binding compared with controls in the striatum and globus pallidus of MPTP monkeys treated with levodopa and cabergoline, a long-acting

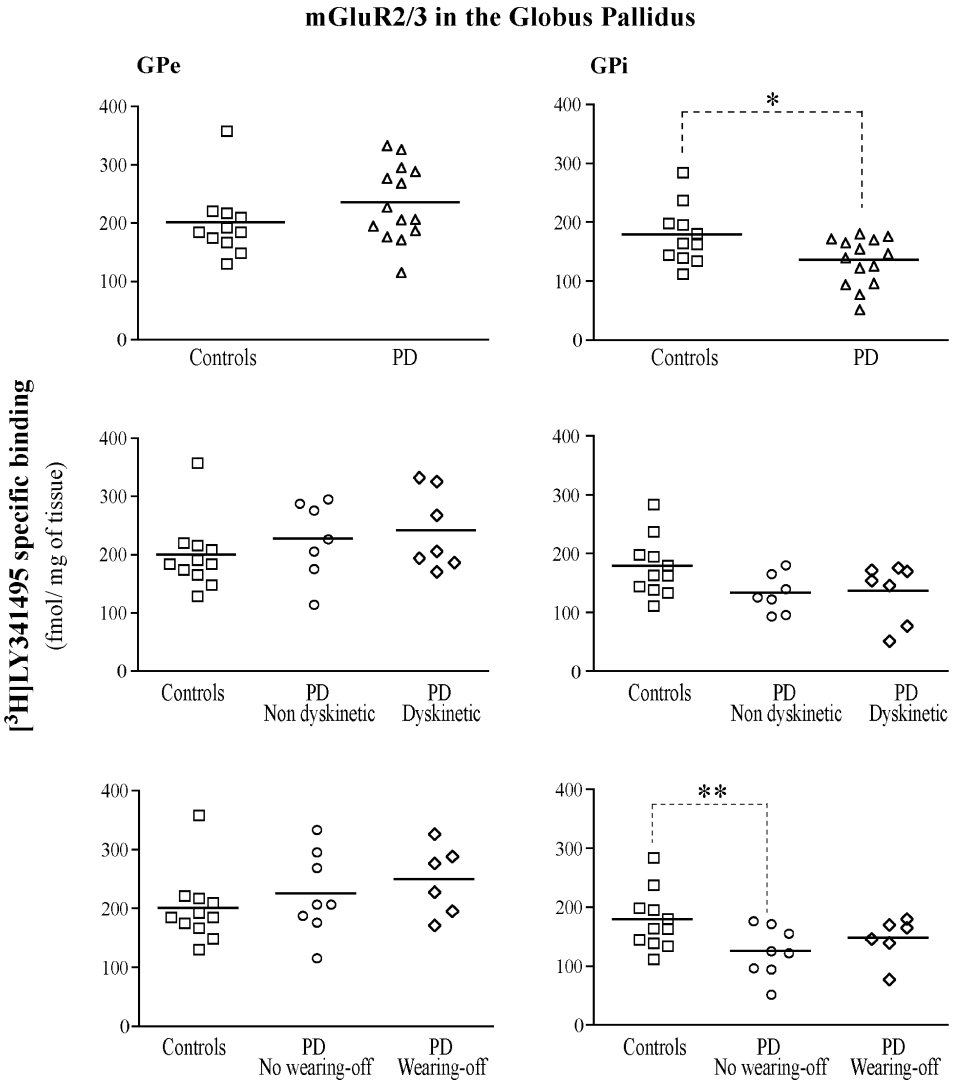

FIGURE 4. [^{3}H]LY341495-specific binding in the external and internal segments of the globus pallidus (external [GPe] and internal [GPi]) of control and levodopa-treated parkinsonian patients. Individual values and means are in femtomoles per milligram (fmol/mg) of tissue. No difference in [^{3}H]LY341495-specific binding was observed in the GPe of Parkinson disease (PD) patients who had or had not experienced motor complications; binding was less in the GPi of PD patients (−25%) compared with controls. This reduction was significant in the GPi of patients who had not developed wearing-off (−30%). *$p < 0.05$ and **$p < 0.01$ vs controls.

dopamine agonist that provides more constant dopaminergic stimulation. This is analogous to the present results in which PD patients who had not had wearing-off had lower caudate nucleus and GPi [^{3}H]LY341495-specific binding; this motor complication is associated with fluctuation of dopaminergic stimulation.

The similar extensive degrees of reduction of striatal dopamine and dopamine metabolites concentrations and the dopamine transporter of the different groups of PD patients (29) precludes differences in striatal denervation as an explanation for motor fluctuations in these patients. The increase of dopamine turnover after nigrostriatal damage (HVA/dopamine) was, however, higher in the putamen of patients with wearing-off than those without wearing-off (29). A higher HVA/dopamine ratio was also reported in the putamen of PD patients with wearing-off compared with patients with dyskinesias or patients without motor complications (35). The association between wearing-off fluctuations and increased dopamine turnover after nigrostriatal damage was observed by positron emission tomography (36). Hence, loss of presynaptic dopamine storage capacity and rapid metabolism of dopamine at striatal synapses may play a relevant role in levodopa-related wearing-off in PD patients (29, 35, 36). This raises the question as to whether the decrease of specific binding to mGluR2/3 in the caudate nucleus is associated with changes in dopamine turnover. Although the HVA/dopamine ratio was not measured in the caudate of these patients, the fact that the specific binding to mGluR2/3 is similar in the caudate nucleus of control subjects and PD patients with wearing-off fluctuations makes this possibility

unlikely. Moreover, wearing-off is also seen with dopamine agonists (which are not stored in presynaptic terminals) (4). No clear correlation was found in positron emission tomography studies of human PD subjects between the degree of damage in dopamine nigrostriatal neurons and motor fluctuations (37).

Our results suggest that in addition to presynaptic inability to buffer levodopa variations, alterations in group II mGluRs responsiveness could also play an important role in the development of motor fluctuations. The mechanisms by which the reduction of group II mGluRs is involved in prevention of motor fluctuations in PD patients are not known; it may have a protective role or there may not be a cause/effect relationship and be secondary to the numerous glutamatergic changes caused by the disease and its treatments.

The differential effect of mGluR2/3 on D1 and D2 dopamine receptors (21) and expression of the latter receptors in the anatomically distinct direct and indirect pathways, respectively (38), suggest that a reduction of mGluR2/3 might contribute to restoring the balance between the striatal output pathways and preventing motor fluctuations in levodopa-treated PD patients. Moreover, activation of mGluR2/3 expressed presynaptically and postsynaptically on striatal cholinergic interneurons can also decrease striatal acetylcholine release (13, 39). Interestingly, the increase of acetylcholine release by the mGluR2/3 antagonist, LY341495, was abolished in dopamine-depleted striatum (40). In PD patients without motor fluctuations, the reduction of mGluR2/3 might contribute to normalization of disrupted striatal cholinergic transmission. Facilitation of striatal acetylcholine release by *N*-methyl D-aspartate receptors (40) and increased specific binding to striatal *N*-methyl D-aspartate receptors of PD patients with wearing-off (6) corroborate this hypothesis.

Why was the reduction of specific binding to mGluR2/3 more pronounced in the caudate nucleus than in the putamen? The caudate nucleus plays a central role in learning a simple stimulus-response association; this is impaired in parkinsonian patients in the ''off'' state and reverts to normal in ''on'' patients treated with levodopa (41, 42). Modifications of dopamine activity in associative territories located in the caudate nucleus could affect attention-deficit behavior (43). Because activation of group II mGluRs induces cognitive and memory deficits (44), the more pronounced reduction of mGluR2/3 receptor binding in the caudate nucleus may act as a compensatory mechanism to prevent motor fluctuations in PD patients.

The striatal medium spiny neurons project to the output nuclei of the basal ganglia, SNr, and GPi monosynaptically or polysynaptically through the GPe and the STN (38, 45). Group II mGluRs are localized presynaptically on STN efferent terminals in the GPi and SNr where they depress glutamate release (17, 46) and postsynaptically in the basal ganglia nuclei (14) where they can regulate neuronal excitability via modulation of ion channel functions (47). Hence, reduction of mGluR2/3 in the GPi of PD patients without wearing-off fluctuations may contribute to restoring the normal GPi neuronal discharge patterns. Moreover, the GPe sends inhibitory projections to the GPi, and the GPe-GPi balance is important in normal neuronal activity of the output basal ganglia circuitry (48). Reduction of functional inhibitory tone of mGluR2/3 on GPe efferent terminals in the GPi, by disinhibition of GPe, could reduce GPi neuronal activity leading to more normalize motor function and improvement of motor fluctuations. Activation of mGluR2/3 to maintain a constant inhibitory tone is hypothesized to suppress locomotor output (49).

In the presence of sufficient intracellular Ca^{2+}, enhanced production of cAMP can be induced by increase of adenosine release after activation of mGluR3 localized in astrocytes (50). This potentiation of cAMP levels is inhibited by adenosine deaminase and adenosine A_{2A} receptor antagonist (50). According to antiparkinsonian effect of A_{2A} receptor antagonist confirmed by behavioral studies in animal models and parkinsonian patients (51, 52), it might be suggested that a reduction of striatal mGluR2/3 may represent a decrease of mGluR3 in astrocytes as a compensatory mechanism to prevent A_{2A} receptor activation and subsequent motor fluctuations. Further investigations are required to elucidate the mechanisms involved in neuron-glial cell interaction in normal and pathological conditions. Our binding assay was based on Wright et al (33), who suggested that under their binding conditions, [^{3}H]LY341495 may bind predominantly to a mGluR3 receptor population in the rat forebrain. Based on the similar distribution pattern of mGluR2 and mGluR3 in preterminal and terminal portions of the axons (19, 53) and stronger expression of mGluR3 in the globus pallidus than mGluR2 (19), it might be speculated that in the striatum and especially in the globus pallidus, our specific binding was mainly to an mGluR3 receptor population. According to the strong labeling of mGluR2 receptor in the caudate and putamen (53), however, we could not rule out the striatal [^{3}H]LY341495-specific binding to mGluR2. The development of subtype-selective radioligands for group II mGluRs is required to decipher mGluR2 and mGluR3 receptor contributions.

Recent studies reported an earlier and higher incidence of motor and nonmotor symptoms of wearing-off, particularly in the earlier stages of the PD compared with the older literature (3, 54, 55). The present study is the first to provide evidence of a decrease of [^{3}H]LY341495-specific binding to mGluR2/3 in the caudate nucleus and GPi of PD patients without wearing-off motor fluctuations. Our results, together with other studies (29, 35, 56), suggest that in addition to presynaptic inability to buffer levodopa variations, alterations in group II mGluR responsiveness may play an important role in the development of motor fluctuations. Increased glutamatergic transmission in PD patients may lead to a compensatory adaptive downregulation of mGluR2/3 in PD patients without wearing-off, and this adaptation may be absent in patients with this motor complication. These results indicate that pharmacological modulation of mGluR2/3 activity might help to manage levodopa-induced wearing-off fluctuations in patients with PD.

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