Increase of Preproenkephalin mRNA Levels in the Putamen of Parkinson Disease Patients with Levodopa-Induced Dyskinesias

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Abstract. The expression of preproenkephalin messenger RNA was studied in the brain of Parkinson disease (PD) patients using in situ hybridization. All these patients were treated with levodopa (LD) and the development of motor complications was recorded. Eleven normal controls and 14 PD patients were used, of which 4 developed dyskinesias, 3 developed wearing-off, 3 developed both dyskinesias and wearing-off, and 4 developed no adverse effect following dopaminomimetic therapy. Nigrostriatal denervation was similar between the subgroups of PD patients as assessed using [125I]-RTI-specific binding to the dopamine transporter and measures of catecholamine concentrations by HPLC. A significant increase of preproenkephalin messenger RNA levels was observed in the lateral putamen of dyskinetic patients in comparison to controls (+210%; p < 0.01) and in comparison to nondyskinetic patients (+112%; p < 0.05). No change was observed in medial parts of the putamen or in the caudate nucleus. No relationship between preproenkephalin messenger RNA levels and other clinical variables such as development of wearing-off, age of death, duration of disease, or duration of LD therapy was found. These findings suggest that increase synthesis of preproenkephalin in the medium spiny output neurons of the striatopallidal pathway play a role in the development of dyskinesias following long-term LD therapy in Parkinson disease.

Key Words: Basal ganglia; Enkephalin; Endogenous opioids; In situ hybridization; Motor complications; Motor fluctuations; Wearing-off.

INTRODUCTION

Levodopa-induced dyskinesias (LID) are, along with "wearing-off" (i.e. a predictable gradual shortening of the duration of action of levodopa [LD] dose), among the main motor complications that accompany long-term dopaminergic treatment in Parkinson disease (PD) (1, 2). LID are involuntary movements of the limb and the trunk that may become as debilitating as are symptoms of PD themselves in a large proportion of LD-treated PD patients (2–4). The role of the basal ganglia in the triggering of dyskinetic behavior is known to be critical (5–7). However, the exact neurochemical mechanism underlying LID remains largely unknown (8).

Three major families of endogenous opioid neuropeptides have been described, the dynorphins, the enkephalins, and the endorphins. Within the human basal ganglia, enkephalins are expressed in medium spiny output neurons of the striatopallidal pathway and play important cotransmitter roles in these GABAergic neurons (9, 10). The precursor gene of enkephalins, preproenkephalin (PPE), encodes [Methionine]enkephalin, [Leucine]enkephalin, [Methionine]enkephalin heptapeptide, and [Methionine]enkephalin octapeptide (11).

Nigrostriatal denervation is associated with an increase of preproenkephalin expression in the striatopallidal neurons in 6-OHDA rats (12–15) and methylphenyltetrahydropyridine (MPTP) monkeys (16–22). These data agree well with the accepted model that dopamine exerts an inhibitory drive on enkephalinergetic neurons and that relief of this inhibitory control induces an increased expression of preproenkephalin (9, 14, 23, 24). Moreover, a relationship between the increase of PPE and the severity of motor impairment has been demonstrated in MPTP monkeys (18).

However, the causal link between expression of PPE and development of motor symptoms associated with dopaminergic depletion has been recently challenged. Indeed, studies in MPTP monkeys and MPTP cats show that an increase of PPE expression can be seen without the appearance of PD symptoms (22, 25). In contrast, PPE expression is turned off to control levels while PD symptoms are still present following long-term MPTP treatment (26).

Postmortem studies in tissue from patients with idiopathic PD have been generally less conclusive that those in animal models. The expression of PPE was measured in the brain of LD-treated PD patients and was shown to be either unaltered (27) or increased in the caudate nucleus and the intermediolateral subregions of the putamen (28). However, these studies in human brains have not taken into account the development of motor complications following LD therapy. Indeed, several lines of evidence suggest that alteration of neuropeptides may be
Preproenkephalin mRNA

Control  Non Dyskinetic  Dyskinetic

levodopa-treated parkinsonian subjects

Fig. 1. Representative autoradiograms of human brain sections at the level of the caudate, putamen, and external and internal segments of the globus pallidus showing preproenkephalin mRNA expression measured by in situ hybridization in control subjects and in LD-treated PD patients with or without dyskinesias.

linked to the pathogenesis of LID (8, 29–31). A study using positron emission tomography using the opioid receptor ligand [11C]diprenorphine shows decreased striatal opioid binding in dyskinetic compared to nondyskinetic PD patients (32). Furthermore, the MPTP-induced increase of PPE mRNA expression found in the lateral caudate and putamen of monkeys was not corrected by LD, and was only partly reversed by a short acting D2 agonist and increased by a short acting D1 agonist (20, 21). All these treatments were also shown to induce dyskinesias (8). Increased expression of PPE mRNA is also seen in caudal striatum of normal monkeys developing dyskinesias after high-dose LD administration (33). In contrast, PPE mRNA expression was reversed to control values in MPTP animals receiving treatment with dopamine agonists inducing a good relief of PD symptoms without dyskinesias (8, 20, 21).

Therefore, in the present study, we have used brain tissue of patients suffering from PD and in whom detailed clinical variables (i.e. age of death, sex, delay to autopsy, pharmacological treatment, age of PD onset, duration of PD, duration of LD use, cumulative LD dose, duration of clinical follow-up, age at LD initiation, duration of PD at the initiation of LD, and average daily dose of LD), as well as the occurrence of motor complications (dyskinesias and wearing-off) have been prospectively recorded by the same neurologist (AHR). Biochemical indices, including brain pH, putaminal dopamine concentration, autoradiography of [125I]-RTI-121-specific binding to dopamine transporter, were also determined. Expression of PPE mRNA was measured using in situ hybridization in an attempt to correlate it with the development of motor complication and the clinical and biochemical variables available.

MATERIALS AND METHODS

Clinical Data

All patients were evaluated by the same neurologist (AHR) at 6 to 12 month intervals as previously described (34, 35). The data, including the age and mode of onset, severity of the disease, drug therapy, response to treatment, and adverse effects of treatment were entered prospectively after each clinical assessment of the patients. All PD patients received LD and some were receiving other antiparkinsonian drugs such as a dopamine
agonist (bromocriptine), amantadine, anticholinergic drugs, or selegiline. These drugs as add-on therapy to LD had no significant effect on the LD-induced motor complication profile of PD patients. Although the amplitude of response after each LD dose declined with time, the wearing-off was classified only when an individual with previous stable response receiving a minimum of 3 LD doses per day experienced predictable motor function decline at the end of the dose. Dyskinesias were identified as involuntary choreic/dystonic movements distinct from the parkinsonian symptoms experienced by the patient. The dyskinesias, although most common at the peak dose, were also noted at other temporal relationships with the LD dose. Patients were divided into groups according to the development of motor complications (Table). These groups were not statistically different in respect to sex, delay to autopsy, age of PD onset, duration of PD, duration of LD use, cumulative LD dose, duration of follow-up, age at LD initiation, duration of PD at the initiation of LD, average daily dose of LD, brain pH, and time delay in the freezer (Table). In the Table, dosage of LD is expressed in equivalent grams of LD only (without decarboxylation) formulation, as previously reported (36).

Autopsy and Handling of the Brain Material

The brains of the 14 PD patients were obtained as well as those from 11 controls (2 from Douglas Hospital Research Center brain bank, Montreal, Canada) who died with no neurolog- ical disorders. The perimortem conditions were comparable in each brain bank, Montreal, Canada) who died with no neurolog- ical disorders. The perimortem conditions were comparable in 24

| TABLE
Clinical and Biochemical Data: Parkinson Disease (PD), Dyskinesias (DK), and Wearing-Off (WO)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age of death (year)</th>
<th>Delay to Autopsy (hours)</th>
<th>Brain tissue pH</th>
<th>Age of PD onset (year)</th>
<th>Duration PD (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>11</td>
<td>68 ± 3</td>
<td>less than 24</td>
<td>6.40 ± 0.07</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PD</td>
<td>14</td>
<td>78 ± 2*</td>
<td>12 ± 2</td>
<td>6.37 ± 0.03</td>
<td>62 ± 4</td>
<td>16.2 ± 2.1</td>
</tr>
<tr>
<td>PD</td>
<td>NON DK</td>
<td>7</td>
<td>80 ± 3</td>
<td>14 ± 2</td>
<td>6.36 ± 0.05</td>
<td>63 ± 3</td>
</tr>
<tr>
<td>PD</td>
<td>DK</td>
<td>7</td>
<td>77 ± 2</td>
<td>11 ± 3</td>
<td>6.39 ± 0.04</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>PD</td>
<td>NON WO</td>
<td>8</td>
<td>78 ± 2</td>
<td>13 ± 3</td>
<td>6.22 ± 0.04</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>PD</td>
<td>WO</td>
<td>6</td>
<td>78 ± 3</td>
<td>12 ± 3</td>
<td>6.44 ± 0.02</td>
<td>60 ± 2</td>
</tr>
</tbody>
</table>

Abbreviations: DK, Dyskinesias; Init, Initiation; LD, Levodopa; PD, Parkinson disease; WO, Wearing-Off. Values are expressed as the mean ± SEM.

* P < 0.01 and ** P < 0.001 vs control.

Biochemistry

Small punch biopsies (15–100 mg) of the cerebral cortex were used for the determination of pH to assess the preservation of the tissue, as previously described (42). The brain slices containing the caudate, putamen, and external and internal globus pallidus from all the subjects were cut into coronal sections (20 μm) on a cryostat (−18°C). The slices were thaw-mounted onto SuperFrostPlus® (Fisher, Nepean, Ontario, Canada) 75 × 50 mm slides, desiccated overnight at 4°C, and stored at −80°C until assayed. In addition, small extracts of putamen were dissected, stored at −80°C, and processed for determination of catecholamine concentrations.

Measurement of Denervation

The concentration of dopamine was measured by HPLC with electrochemical detection according to previously published procedures (43). The dopamine transporter was evaluated with [3H]-RTI-121- (3β-(4-[125 I]-iodophenyl)tropane-2β-carboxylic acid isopropyl ester) (2,200 Ci/mmol; NEN-DuPont, Boston, MA) specific binding according to a previously published procedure in human brain sections (44). Details on the methodology used in this section are reported elsewhere (45).

In Situ Hybridization

In situ hybridization procedures were performed essentially as described by Wisden and Morris (46). A 48-mer oligonucleotide was used corresponding to bases 284–331 of human PHE-A cDNA according to previous studies in humans (11) and monkeys (20, 21). The expression of the “house-keeping” control gene β-actin was measured in adjacent sections using 3 oligonucleotides corresponding to bases 77–121, 293–337, and 931–975 according to published cDNA sequences (47). Oligonucleotides were labeled with [35 S]-dATP (NEN-DuPont) using a 3'-terminal deoxynucleotidyltransferase enzyme kit (Amersham Pharmacia Biotech, Baie d’Urfé, Quebec, Canada). The reaction was carried out at 37°C for 40 min and labeled oligonucleotides were purified on NENsorb 20 cartridges (NEN-DuPont) and 0.01 M DTT was added to the purified probe, which was kept

at −20°C until the assay on the next day (46). After drying under vacuum with a desiccant (4°C), the sections were fixed for 5 min in 4% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA) prepared in 0.1 M sodium phosphate buffer (PBS), pH 7.4, at room temperature and then rinsed twice for 5 min in PBS at room temperature. The sections 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. They were then rinsed (2 min) twice in 2 × SSC (standard saline citrate: 1 × SSC is 0.15 M NaCl, 0.015 M trisodium citrate, pH 7.0) and dehydrated through a series of ascending concentrations of ethanol (70%, 85%, and 95%, 1 min each), air-dried, and stored for 2 to 3 h under vacuum with desiccant at room temperature. In addition, a few sections were hybridized in the presence of a 100-fold excess of unlabeled probe to displace specific labeling.

The oligonucleotide probe(s) was diluted (5 × 10⁶ cpm/ml) in the hybridization buffer containing 50% deionized formamide, 10% dextran sulfate, 1 × Denhardt’s solution, 0.25 mg/ml yeast tRNA, 0.5 mg/ml denatured salmon sperm DNA, and 4 × SSC. Hybridization was performed at 40°C for 18 h in a humid chamber with each slide covered with a glass coverslip. Sections were then washed successively in 2 × SSC (90 min at room temperature), 1 × SSC (120 min at room temperature), 0.5 × SSC (30 min at 42°C), 0.5 × SSC (30 min at room temperature), 0.5 × SSC (30 min at 50°C). Finally, the slides were dehydrated in a series of ascending concentrations of ethanol (70%, 85%, and 95%, 1 min each), air-dried, and exposed to Kodak BIOMAX MR film for 14 days at room temperature along with standards (¹⁴C-micro-scales, Amersham). Hybridization products were obtained from Sigma (St. Louis, MO).

Quantification of all autoradiograms was performed on a power Macintosh 7100 connected to a Sony video camera (model XC-77) and a constant illumination fight table using computerized densitometry with the software package NIH Image 1.61. Optical gray densities were transformed into fmol/mg of tissue equivalent using standard curve generated with standards. The results were then converted into fmol/mg of tissue using the estimated specific activity of the oligonucleotide probe (=1250 Ci/mmol). Nonspecific signal, as assessed with excess of unlabeled probe, was subtracted from these values.

For analysis, caudate nucleus and putamen were divided into 2 subregions along a medial-lateral axis (Fig. 2A). Data were computed separately for each subregion and were grouped when regional effects were similar. Statistical comparisons of the data were performed using an ANOVA followed by post-hoc pairwise comparisons with Fisher’s probability of least significant difference test (PLSD). A value of p < 0.05 was judged significant. First, the 11 Control subjects were compared to the 14 Parkinsonian subjects. In subsequent analysis, comparisons were made between controls, Parkinsonian nondyskinetic subjects, and Parkinsonian dyskinetic subjects. Alternatively, Controls were compared to Parkinsonian without wearing-off and Parkinsonian with wearing-off in a different ANOVA. Coefficients of correlation and significance of the degree of linear relationship between various clinical and biochemical parameters were determined with a simple regression model. All correlations were made independently for controls, PD patients and PD patients with dyskinesias.

### RESULTS

The comparisons of clinical data between the different groups of subjects are shown in the Table. No relation between clinical data and the development of wearing-off and dyskinesias was observed; although, Parkinsonian

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**TABLE (extended)**

<table>
<thead>
<tr>
<th>Duration LD use (year)</th>
<th>Cumulative LD dose (g)</th>
<th>Duration PD at LD init. (year)</th>
<th>Daily LD dose (g)</th>
<th>[¹²⁵I]RTI-121 specific binding (amol/mg tissue)</th>
<th>Dopamine concentration (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>~</td>
<td>~</td>
<td>~</td>
<td>~</td>
<td>910 ± 81</td>
<td>63.21 ± 3.97</td>
</tr>
<tr>
<td>11.2 ± 1.6</td>
<td>13651 ± 3413</td>
<td>4.2 ± 0.9</td>
<td>3.2 ± 0.6</td>
<td>69 ± 6**</td>
<td>0.77 ± 0.14**</td>
</tr>
<tr>
<td>11.9 ± 2.8</td>
<td>15316 ± 6422</td>
<td>5.3 ± 1.5</td>
<td>2.8 ± 0.7</td>
<td>70 ± 10</td>
<td>0.72 ± 0.22</td>
</tr>
<tr>
<td>10.7 ± 1.8</td>
<td>11984 ± 2884</td>
<td>3.1 ± 1.1</td>
<td>3.7 ± 0.9</td>
<td>69 ± 9</td>
<td>0.82 ± 0.17</td>
</tr>
<tr>
<td>11.6 ± 2.2</td>
<td>11936 ± 3765</td>
<td>3.0 ± 0.9</td>
<td>2.4 ± 0.4</td>
<td>67 ± 9</td>
<td>0.71 ± 0.17</td>
</tr>
<tr>
<td>10.9 ± 2.5</td>
<td>15937 ± 6511</td>
<td>5.8 ± 1.7</td>
<td>4.3 ± 1.0</td>
<td>72 ± 9</td>
<td>0.84 ± 0.24</td>
</tr>
</tbody>
</table>

**Fig. 2.** Schematic representation of the caudate, putamen, and external and internal segment of the globus pallidus, showing in panel (A), the actual division used for quantification of autoradiograms and in panel (B), the localization of associative (AS), sensorimotor (SM), and limbic (LI) striatal territories in primates. Abbreviations: CD, caudate; IC, internal capsule; GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; PUT, putamen; L, lateral; M, medial.
subjects were older than controls. Correlation between age of death and preproenkephalin mRNA expression was studied and no significant correlation was found (Fig. 4). An extensive decrease of DA (~98.8%) was found in the putamen of Parkinsonian patients consistent with the diagnosis of advanced PD (Table). Moreover, an important decrease of 125I-RTI-121-specific binding was also observed in the putamen (~92%) of Parkinsonian patients (Table). Dyskinesias and/or wearing-off were not associated with different levels of nigrostriatal denervation as assessed by catecholamine measurements and 125I-RTI-121-specific binding to dopamine transporters (Table). Brain pH was comparable between each group of subjects used in the present study (Table).

The expression of preproenkephalin mRNA was increased (+127%, p = 0.0497) in the lateral part of the putamen in PD patients (Figs. 1, 3). However, important
individual variability was noted. Interestingly, the increase of PPE mRNA expression in the lateral part of the putamen was more pronounced in PD patients suffering from LID (Figs. 1, 3). The increase was statistically significant in comparison to nondyskinetic (+112%; p = 0.0424) and in comparison to the controls (+210%; p = 0.0059). No alteration of PPE mRNA expression was seen in the caudate and in the medial putamen of dyskinetic patients (Fig. 3). The development of wearing-off was not significantly correlated with alteration in PPE mRNA expression but a trend towards an increase was observed throughout the striatum (Fig. 3). In contrast, there was no significant difference in levels of β-actin mRNA (which was used as a control mRNA) between the subgroups studied (data not shown). No interrelation was found between PPE mRNA expression and age of death, duration of PD, or the duration of LD treatment in PD patients (Fig. 4). No correlation was observed between PPE mRNA expression and age of death in controls. No relationship was found between PPE mRNA expression and gender, delay to autopsy, time delay in the freezer, age of PD onset, cumulative LD dose, duration of follow-up, age at LD initiation, duration of PD at the initiation of LD, average daily dose of LD, or the medical treatment (data not shown). In the putamen, a significant correlation between PPE mRNA expression and brain pH was observed in the controls (n = 9, r² = 0.673, p = 0.0067) but not in PD patients (n = 14, r² = 0.026, p = 0.5818) or control and PD patients together (n = 23, r² = 0.087, p = 0.1724). A similar correlation between PPE mRNA expression and brain pH was found in the caudate in these groups (data not shown).

DISCUSSION

The main evidence of altered peptidergic activity in LID in PD patients was discovered by Piccini et al using positron emission tomography. A downregulation of striatal and thalamic [11C]diprenorphine binding to opioid receptor was shown in patients with LID in comparison with other patients devoid of this adverse effect (32). This interesting observation is consistent with peptidergic overactivity in LID. Indeed, diprenorphine binds to δ, κ, and μ opioid receptor subtypes, and a decreased number of these receptors may be explained by a compensatory mechanism following increase release of neuropeptides (32). However, this result did not differentiate between overactivity of enkephalin, which binds to δ or μ receptors, or of dynorphin, which binds mainly to κ receptors (48, 49).

Previous studies in MPTP monkeys have shown that alteration of PPE mRNA expression may underlie the pathogenesis of LID. Indeed, the induction of parkinsonism in monkeys with the neurotoxin MPTP provides an exceptional model for the study of dyskinesias by dopamine-like agents (8, 50). In this model, MPTP-induced denervation is associated with an increase of striatal PPE mRNA expression in comparison to normal controls (16–21). Dopamine agonist treatment that did not induce dyskinesias corrected the MPTP-induced increase of PPE mRNA expression whereas dyskinesiogenic treatment with LD or a dopamine agonist did not (20, 21, 29). Therefore, the dyskinesiogenic potential of dopaminomimetic drugs in MPTP monkeys may be linked to its inability to reverse the increase of PPE mRNA expression, which follows nigrostriatal denervation (29). Furthermore, in normal monkeys, LID are also associated with an increase of PPE mRNA expression (33).

The studies in MPTP monkeys drawing a link between dyskinesias and PPE expression investigated the acute effect of MPTP and dyskinesias following a short 1-month treatment (20, 21). This is different from idiopathic PD, in which patients suffer from a progressive illness and LID usually appears insidiously after years of LD treatment. In this regard, no increase of PPE expression was found 8 months after the last dose of a chronic MPTP administration protocol expected to more closely mimic PD evolution (26). This observation would suggest that the increase of PPE expression is an acute reaction to dopamine depletion, and that it is slowly reversed over time despite the motor symptoms still present (26). Nevertheless, in the present study, the dyskinetic patients had suffered from PD for an average of 16 yr and had received LD for more than 10 yr. Clearly, the duration of the disease or the duration of LD therapy was not correlated with a decrease of PPE mRNA expression in PD. These observations and the increased PPE mRNA expression observed in dyskinetic patients with an advanced disease suggest that alterations in PPE synthesis are long lasting in human idiopathic PD.

Interestingly, the increase of PPE mRNA expression in dyskinetic patients was restricted to the lateral parts of the putamen and was not detectable elsewhere in the striatum. Accordingly, lateral parts of the putamen integrate information mostly from cortical projection originating from the sensorimotor cortex areas (Fig. 2B). In agreement with the present data, the induction of PPE mRNA expression observed in human parkinsonian patients, MPTP monkeys, and 6-OHDA rats are mostly observed in sensorimotor area of the striatum (17, 20, 21, 28, 51, 52). It is known that glutamate-releasing projections from the cortex exert a positive control on the regulation of PPE synthesis in animal models of PD (53). Accordingly, lesion of the corticostratial pathway (54) or blockade of NMDA receptors by systemic injection of MK-801 (55) induces a decrease in PPE mRNA expression in the striatum of intact rats. Therefore, LID may be related to an upregulation of enkephalin synthesis in the lateral putamen caused by a dysfunction (increased glutamate release?) in the corticostratial pathways from the motor cortex. Indeed, there are several pieces of evidence...
suggesting that a pathological increase in glutamatergic neurotransmission in the striatum plays a role in the pathogenesis of LID and that glutamate receptor antagonists have an antidysonkinesiogenic profile (56–62).

Several transcription factors regulate PPE mRNA expression and could be involved in the present PPE mRNA upregulation (63). For example, ΔFosB, which accumulates following several types of chronic perturbations, can form an AP-1 (activator protein 1) complex with a Jun-like protein and modulate the transcription of PPE through its binding on a AP-1 consensus site or a CRE (cAMP response element) site located on the PPE gene (29, 63, 64). Indeed, ΔFosB immunoreactivity and PPE mRNA expression are both increased in dyskinetic monkeys following a D1 agonist treatment (21, 29, 65). Adenosine receptors located on striatopallidal neurons also contribute to the regulation of expression of PPE mRNA in dopamine-depleted striatum (66, 67). The mechanism of this regulation may involve alteration of intracellular cAMP concentration or c-fos-related pathway of cellular activation (67). Interestingly, antagonists of A2A receptor reduce dyskinesias in LD-treated MPTP monkeys (68, 69). Consequently, it is tempting to speculate that the antidysonkinesiogenic properties of A2A receptor antagonists involve a downregulation of PPE mRNA expression.

The present data also reveal important interindividual variability in the level of expression of the PPE mRNA transcript. Different clinical or biochemical indices that could account for this variability or that could be potential confounding variables have been investigated. Previous studies in postmortem human brain samples show that a decrease of brain pH is associated with prolonged agonal state and degradation of mRNA products (42, 70, 71). Indeed, when brain pH is low, the mRNA content of PPE and other ubiquitous peptides in human brain are expected to be reduced (42). The present results confirm the correlation between brain pH and PPE mRNA expression in the caudate/putamen of control subjects. However, that correlation was not found in the 14 PD patients included in the study, suggesting that other factors may be more important in these cases. The influence of decreased pH is probably not a confounding variable in the present study since all groups were pH-matched. Other variables contingent upon the quality of the tissue used, such as postmortem delay and time in the freezer, were tested and no correlation was found. Since the controls included in the present study were younger than PD subjects, the effect of age on PPE expression data was investigated. No relationship was determined, suggesting that age of death is not a relevant confounding factor here.

Enkephalin is a neuromodulator that is thought to exert an inhibitory control on the release of GABA in the GPe through its interaction with opioid (δ and μ) receptors located on striatopallidal axons and/or GPe neurons (72–74). Increased enkephalinergic/GABAergic transmission ratio in the GPe may result in a reduction of GABA release in the GPe and disinhibition of GPe neurons. According to the classical model of basal ganglia function in hyperkinetic disorders, disinhibition of GPe neurons will lead to decreased firing rate of GPi towards the thalamus and consequent disinhibition of the thalamocortical circuit thought to generate dyskinesias (6, 7, 31, 75). Indeed, electrophysiological studies show that most GPi neurons are less active in dyskinesias (5, 76–79). This interpretation is in accordance with the hypothesis of Henry and Brotchie that suggested that enkephalinergic overactivity in the GPe may play a role in the pathogenesis of LID (31). Supporting these data, direct injection of μ receptor agonists in the globus pallidus of rats stimulates oral dyskinesias (80). However, the exact role of the GPe in dyskinesias is still elusive and may not conform to previous models of basal ganglia function, on which the present interpretation is based (5).

The increased production of new PPE mRNA probably reflects an accelerated synthesis of PPE in dyskinesias. However, this does not prove that there is an increased release of enkephalin at the end of the striatopallidal axons. These neurons are known to project mainly to the GPe, but recent studies show that these neurons have highly collateralized axons extending to other areas of the striatum and to the GPi and substantia nigra (81, 82).

However, in vitro measurement of enkephalin content in the caudate/putamen and the globus pallidus of MPTP monkeys and PD patients have generally brought inconsistent results. In the striatum, studies of the enkephalin content in postmortem tissue from human brains with PD report either no change (83, 84) or a decrease of [methionine]enkephalin in the putamen (85–87) and of [leucine]enkephalin in the putamen (85, 86). The decrease of [methionine]enkephalin seen in the caudate nucleus tends to correlate with disease severity and nigrostriatal denervation, but shows interindividual variability in PD patients (83, 88). In the GPe, [methionine]enkephalin is either increased (89, 90), unchanged (85, 91), or reduced (86) in PD depending of the experiment. In human PD, [leucine]enkephalin levels are unchanged (85) or decreased in the GPe (86).

Radioimmunoassay experiments performed in samples from the caudate and putamen of MPTP monkeys show either a robust decrease of [methionine]enkephalin levels (92), no change of [methionine]enkephalin and [leucine]enkephalin concentrations (93–96), or unaltered [Methionine]enkephalin content associated with an increase in its biosynthesis (97). In another study, the immunoreactivity for enkephalin was enhanced in the sensorimotor territory of the striatum of MPTP monkeys (98). In the GPe, an increase of [methionine]enkephalin immunoreactivity parallels the severity of parkinsonism in MPTP monkeys (96). However, other studies do not
find an alteration of [methionine]enkephalin and [leucine]enkephalin in the GPe of common marmosets following MPTP treatment (94, 95). These inconsistencies, which are partly explained by variations in the technical approaches, in the exact level of dopaminergic depletion and in time after the lesion preclude any clear appreciation of the enkephalinergic activity in the striatopallidal complex. However, studies on levels of neuropeptides in human PD were not put in parallel with the development of LD-induced motor complications and are difficult to interpret in relation with the present results. Further studies such as in vivo microdialysis in animal model of PD are needed to investigate the correlation between changes of PPE mRNA and proenkephalin or enkephalin levels.

Opioid receptor antagonists have been proposed as adjuncts to standard dopaminergic drugs in order to reduce the problem of LID in LD-treated PD patients (30, 31). This hypothesis is based on the assumption that blockade of enkephalinergic transmission in the globus pallidus may have an antidyskinetic action and is supported by the present results (30, 31). Small clinical trials with the opioid receptor antagonist naltrexone tend to support this issue (99, 100). However, low doses of naltrexone (0.02–0.04 mg/kg) do not have antidyskinetic effects in MPTP monkeys (101) and negative results were obtained with naltrexone in clinical studies in PD patients (102, 103). Intriguingly, low doses of an opioid receptor agonist morphine was recently shown to reduce LID without decreasing the antiparkinsonian response (104). Overall, opioid receptor antagonists are an interesting avenue for LID treatment, but several questions on the dose-response curve of opioid receptor antagonists and the exact receptor subtype that should be targeted need to be answered.

The present observations suggest that therapeutic interventions that would block the expression of PPE in the putamen may be a relevant antidyskinesiogenic strategy. For example, antisense therapeutic targeting the PPE mRNA sequence may be an interesting research avenue. However, no causal link may be ascertained from the present association between increased PPE expression and dyskinesias. Indeed, increased PPE expression may be a compensatory mechanism triggered by pathological information processing in the corticostratal loop that is part of a complex cascade of molecular events. It may not be directly linked to LID per se and may even be an adaptive mechanism induced by the brain to disrupt the dyskinetic process. Interestingly, electrophysiological data suggests that the main effect of opioid in striatal neurons is to inhibit corticostratal excitatory input (105). Hence, increase in preproenkephalin expression could be part of a feedback loop intended to rectify the altered glutamatergic transmission that might be involved in the pathogenesis of LID.

Conclusion

Several conclusions can be drawn from the present data: 1) Dyskinesias produced by chronic LD treatment (Mean duration: 10.7 ± 1.8 yr) are associated with an increased preproenkephalin mRNA expression in the lateral putamen of PD patients. 2) The increase of preproenkephalin mRNA expression is restricted in the sensorimotor area of the putamen. 3) Levels of expression of preproenkephalin mRNA were not correlated with any other important clinical variables such as the age of death, the duration of the disease, or the duration of LD therapy. 4) Overall, these data suggest that there is a critical link between an upregulation of the synthesis of preproenkephalin mRNA and the development of LD-induced dyskinesias in PD patients treated with LD for several years.

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