Increased adenosine A2A receptors in the brain of Parkinson’s disease patients with dyskinesias

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Summary
Brain adenosine A2A receptors have recently attracted considerable attention because of their interaction with the dopaminergic system and as potential targets for Parkinson’s disease pharmacotherapy. Post mortem adenosine A2A receptor mRNA and [3H]SCH 58261-specific binding to adenosine A2A receptor were studied in the brain of Parkinson’s disease patients using in situ hybridization and receptor binding autoradiography, respectively. Fourteen levodopa-treated Parkinson’s disease patients, of which seven developed dyskinesias and seven did not, were compared with nine controls. Nigrostriatal denervation was similar between dyskinetic and non-dyskinetic Parkinson’s disease patients, as assessed with catecholamine concentrations and [125I]RTI-121-specific binding to dopamine transporters. A2A receptor mRNA levels (+129%; P < 0.01) and [3H]SCH 58261-specific binding (+32%, P < 0.01) were increased in the putamen (lateral and medial) of dyskinetic patients compared with controls. The increase of adenosine A2A receptor mRNA in dyskinetic Parkinson’s disease patients was also significant compared with non-dyskinetic Parkinson’s disease patients (+60%; P < 0.05) in the lateral putamen. Moreover, [3H]SCH 58261-specific binding to adenosine A2A receptors was increased in the external globus pallidus (+24%; P < 0.001) of Parkinson’s disease patients compared with controls, regardless of the dyskinesigenic response to levodopa. No change of adenosine A2A receptors was observed in the caudate nucleus, whereas adenosine A2A receptor protein and mRNA levels in the internal globus pallidus were not different from background. These findings suggest that increased synthesis of adenosine A2A receptors in striatopallidal pathway neurons is associated with the development of dyskinesias following long-term levodopa therapy in Parkinson’s disease.

Keywords: adenosine A2A receptors; motor complications; Parkinson’s disease; putamen; SCH 58261

Abbreviations: AP = activator protein; A2AR = adenosine A2A receptor; GPe = external segment of the globus pallidus; LID = levodopa-induced dyskinesias; MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; OHDA = hydroxydopamine; PPE = preproenkephalin

Introduction
Despite marked efficiency in the treatment of Parkinson’s disease symptoms, sustained therapy with levodopa and dopamine agonists also induces motor complications such as levodopa-induced dyskinesias (LID) (Fahn, 2000; Obeso et al., 2000a; Rajput et al., 2002). These motor complications limit considerably the pharmaceutical care of Parkinson’s disease patients, as they can be as debilitating as the Parkinson’s disease symptoms.

One of the most attractive strategies for treating LID is to use adjunct pharmacological tools that can modulate...
non-dopaminergic neurotransmitter systems in the basal ganglia, such as cannabinoids, GABA, neuropeptides, glutamate and adenosine (Brotchie, 1998, 2003; Blanchet et al., 1999; Grondin et al., 1999; Henry et al., 2001; Calon and Di Paolo, 2002). Drugs interacting with receptors involved in these systems could then be used in combination with levodopa to improve the motor response and dyskinetic profile of levodopa alone. In this regard, adenosine A2A receptor (A2AR) has received increasing attention recently as a potential pharmacological target because of the close interaction between A2AR and the dopaminergic systems (Ferre et al., 2001).

A2AR are G protein-coupled receptors that are predominantly found in the GABAergic striatopallidal neurons projecting from the caudate nucleus and the putamen, mainly to the external segment of the globus pallidus (indirect pathway) (Martinez-Mir et al., 1991; Schiiffmann et al., 1991; Svenningsson et al., 1999; Kase, 2001). In primates, this group of neurons coexpress preproenkephalin (PPE), glutamic acid decarboxylase (GAD) and D2 receptors along with A2AR (Svenningsson et al., 1998; Aubert et al., 2000), and play an important role in the pathogenesis of Parkinson’s disease symptoms and LID (Richardson et al., 1997; Crossman, 2000; Jenner, 2000; Obeso et al., 2000b; Calon et al., 2002). Owing to the recent development of specific agonists and antagonists to A2AR, it is now becoming possible to selectively target striatopallidal neuron function in motor behaviour with limited effects on other components of the brain.

Several behavioral analyses demonstrate the potential usefulness of A2AR antagonism in the treatment of Parkinson’s disease and LID. Studies in non-human primate models of Parkinson’s disease suggest that the A2AR antagonist KW-6002 has an antiparkinsonian activity and potentiates the activity of levodopa (Kanda et al., 1998; Grondin et al., 1999; Kanda et al., 2000). More interestingly, these effects are seen without exacerbation of dyskinesias (Grondin et al., 1999; Kanda et al., 2000). Comparable effects of A2AR antagonists on levodopa-induced motor complications are found in rats with a unilateral 6-hydroxydopamine (OHDA)-induced dopaminergic lesion (Morelli and Pinna, 2001; Pinna et al., 2001; Bove et al., 2002; Bibbiani et al., 2003; Lundblad et al., 2003). Moreover, behavioral sensitization to repeated levodopa administration is markedly reduced in transgenic mice lacking the A2AR gene (Fredduzzi et al., 2002). Although definite data on antidyskinetic propriety of A2AR antagonists are still unavailable in human, a recent study showed that KW-6002 potentiates the beneficial effect on parkinsonian symptoms of a low dose of levodopa, with a only mild increase in dyskinesias severity (Bara-Jimenez et al., 2003). The non-selective adenosine antagonist theophylline induced significant motor improvement and slightly increased ON time in small open-label clinical studies in Parkinson’s disease patients (Mally and Stone, 1994; Kostic et al., 1999). However, theophylline failed to modulate the response to levodopa in a more recent double-blind, crossover, placebo-controlled trial in Parkinson’s disease patients (Kulisevsky et al., 2002).

The frequency of LID in Parkinson’s disease patients varies according to the studies, but ranges between 20 and 50% of patients after years of treatment in most recent reports (Blanchet et al., 1996; Miyawaki et al., 1997; Rascol et al., 2000; Ahlskog and Muenter, 2001; Rajput et al., 2002). These studies and clinical experience also show that a small but significant proportion of Parkinson’s disease patients do not develop dyskinesias, despite prolonged therapy with levodopa. We have used post mortem brain tissue of patients who had suffered from Parkinson’s disease and in whom detailed clinical variables (age of death, sex, delay to autopsy, pharmacological treatment, age of Parkinson’s disease onset, duration of Parkinson’s disease, duration of levodopa use, cumulative levodopa dose, duration of clinical follow-up, age at levodopa initiation, duration of Parkinson’s disease at the initiation of levodopa and average daily dose of levodopa), as well as the occurrence of motor complications (dyskinesias and wearing-off), had been prospectively recorded by the same neurologist (A.H.R.) (Calon et al., 2002; Rajput et al., 2002). Biochemical indices (brain pH, putaminal dopamine concentration, autoradiography of [125I]RTI-121-specific binding to dopamine transporter and expression of PPE mRNA) were also determined previously (Calon et al., 2002, 2003). Using post mortem brain tissue from these patients and controls, we have addressed the question of whether changes of A2AR are associated with LID, by comparing patients who suffered from LID with patients who never developed this adverse effect.

**Material and methods**

**Clinical data**

The patients included in this study have been described previously (Calon et al., 2002, 2003) and were selected from a large prospective study on motor complications associated with levodopa treatment (Rajput et al., 2002). Briefly, data including the age and mode of onset, severity of the disease, drug therapy, response to treatment and adverse effects of treatment (dyskinesias, wearing-off and on-off) were entered prospectively after each clinical assessment of the patients (Rajput et al., 2002). LID were evaluated by the same neurologist (A.H.R.) in all patients. The presence of dyskinesia was based on: (i) history of abnormal movements (as distinct from tremor) that coincided with levodopa ingestion, reported by the family/other observer or the family physician. This information was considered valid only if it was confirmed by the neurologist during personal interview with the patient or family at subsequent assessment; or (ii) evidence of choreic/dystonic movements observed at the time of examination by the neurologist. Any patient who was documented to have dyskinesias on one occasion was classified as having LID, regardless of whether subsequent therapeutic manipulations resolved the dyskinesias (Rajput et al., 2002). All Parkinsonian patients received levodopa and were divided into groups according to the development of motor complications (Table 1). These groups were not statistically different with respect to sex, delay to autopsy, terminal Parkinson’s disease severity, as
assessed with the Hoehn and Yahr scale (Hoehn and Yahr, 1967; Rajput et al., 2002), age of Parkinson’s disease onset, duration of Parkinson’s disease, duration of levodopa use, cumulative levodopa dose, duration of follow-up, age at levodopa initiation, duration of Parkinson’s disease at the initiation of levodopa, average daily dose of levodopa, timing of last dose of levodopa before death, brain pH and time delay in the freezer (Table 1 and data not shown). The dosages of levodopa in Table 1 are expressed in equivalent in grams and time delay in the freezer (Table 1 and data not shown). The variations in the autopsies were carried out within 24 h of death. One half of the brain was immediately frozen at −80°C until assayed. In each case, a few milligrams of tissue was excised from the putamen, cut into small pieces and stored at −80°C until assay. The other half of the brain was cut into coronal sections (20 μm) on a cryostat (−18°C). The slices were thaw-mounted onto SuperFrostPlus™ (Fisher, Quebec, 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 measures of catecholamine concentrations.

Table 1  Relevant clinical and biochemical data in relation to Parkinson's disease and LID

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age at death (years)</th>
<th>Delay to autopsy (h)</th>
<th>Brain tissue (pH)</th>
<th>Age of PD onset (years)</th>
<th>Duration of PD (years)</th>
<th>Duration of levodopa use (years)</th>
<th>Cumulative levodopa dose (g)</th>
<th>Putamen [125I]RTI-121-specific binding (amol/mg tissue)</th>
<th>Dopamine concentration (ng/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>9</td>
<td>68 ± 3</td>
<td>&lt;24</td>
<td>6.37 ± 0.07</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>830 ± 68</td>
<td>63.87 ± 4.89</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</td>
<td>11.2 ± 1.6</td>
<td>13 651 ± 3413</td>
<td>69 ± 6**</td>
<td>0.77 ± 0.14**</td>
</tr>
<tr>
<td>PD, LID</td>
<td>7</td>
<td>80 ± 3</td>
<td>14 ± 2</td>
<td>6.36 ± 0.05</td>
<td>63 ± 3</td>
<td>17.4 ± 3.5</td>
<td>11.9 ± 2.8</td>
<td>15 316 ± 6422</td>
<td>70 ± 10</td>
<td>0.72 ± 0.22</td>
</tr>
<tr>
<td>PD, no LID</td>
<td>7</td>
<td>77 ± 2</td>
<td>11 ± 3</td>
<td>6.39 ± 0.04</td>
<td>61 ± 2</td>
<td>15.4 ± 2.4</td>
<td>10.7 ± 1.8</td>
<td>11 984 ± 2884</td>
<td>69 ± 9</td>
<td>0.82 ± 0.17</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. *P < 0.01 and **P < 0.001 versus controls. PD = Parkinson’s disease; LID = levodopa-induced diskinesias.

Measures of denervation

The concentration of dopamine was measured by high-performance liquid chromatography with electrochemical detection according to previously published procedures (Calon et al., 2003). The dopamine transporter was evaluated with [125I]RTI-121 [3β-(4-[125I]-iodo-phenyl)tropane-2β-carboxylic acid isopropyl ester] (2200 Ci/mm; NEN-DuPont, Boston, MA, USA) specific binding according to previously published procedures in human brain sections (Staley et al., 1995; Calon et al., 2003).

In situ hybridization

The oligonucleotide probes used corresponded to bases 593–637 and 714–757 of human A2AR cDNA (Schiffmann et al., 1991; Furlong et al., 1992). Three oligonucleotides corresponding to bases 77–121, 293–337 and 931–975 according to published cDNA sequences were used to assess the β-actin mRNA content in adjacent sections (Ponte et al., 1984). Oligonucleotides were labelled with [35S]dATP (NEN-DuPont) using a 3'-terminal deoxynucleotidyl transferase enzyme kit (Amersham-Pharmacia Biotech, Baie d’Urfé, Québec, Canada). The reaction was carried out at 37°C for 40 min and labelled oligonucleotides were purified using a QIAquick Nucleotide Removal Kit (Qiagen Inc., Québec, Canada). The purified probe was kept at −20°C until the assay on the next day.

After drying under vacuum with a desiccant (4°C) for 2 h, the sections were fixed for 5 min in 4% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA, USA) prepared in 0.1 M sodium phosphate buffered-saline (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× standard saline citrate (SSC; 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 2–3 h under vacuum with desiccant at room temperature. In addition, a few sections were hybridized in the presence of a 100-fold excess of unlabelled probe to displace specific labelling.

The oligonucleotide probe mixtures were diluted (5 ± 10pm/ml) in the hybridization buffer containing 50% deionized formamide, 10% dextran sulphate, 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.

Adenosine A2A receptors in Parkinson’s disease

Small punchers (15–100 mg) of the cerebral cortex were used for the determination of pH, as previously described, to assess the preservation of the tissue (Kingsbury et al., 1995). The brain slices containing the caudate, putamen, 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, Quebec, 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 measures of catecholamine concentrations.
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, 42°C), 0.5× SSC (30 min at room temperature) and 0.5× SSC (30 min, 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. Hybridization products were obtained from Sigma (St Louis, MO, USA).

**[3H]SCH 58261 binding autoradiography**

A2AR were evaluated using a high-affinity selective antagonist, [3H]SCH 58261 (77 Ci/mmol; Schering Plough, Kenilworth, NJ, USA), according to previously published procedures (Dionisotti et al., 1997; Svenningsson et al., 1997). Brain sections were preincubated for 30 min in a 170 mM Tris–HCl buffer (pH 7.4) with 2 U/ml adenosine deaminase (Roche Diagnostics Corp., Indianapolis, IN, USA) and 1 mM EDTA (Sigma) at room temperature to remove endogenous adenosine. After two additional 20 min preincubations in 170 mM Tris–HCl buffer (pH 7.4), the sections were incubated for 120 min at room temperature with 2 nM [3H]SCH 58261 in 170 mM Tris–HCl (pH 7.4) with 2 U/ml adenosine deaminase, and then washed twice (5 min) in 170 mM Tris–HCl buffer (pH 7.4) at 4°C and rinsed briefly (10 s) in ice-cold distilled water. Non-specific binding was determined by adding 2 × 10−4 M 5′-ethylcarboxamidoadenosine (RBI Inc, Natick, MA, USA) to the incubation buffer. After postincubation washes, the slide-mounted tissue sections were dried overnight at room temperature and then exposed to [H]-sensitive films (Hyperfilm; Amersham, Baie d’Urfé, Quebec, Canada) along with tritium standards ([H]-microscales, Amersham) over 8 weeks.

**Image, data and statistical analysis**

Quantitation of all autoradiograms was performed on a power Macintosh 7100 connected to a Sony video camera (model XC-77) and a constant illumination light table using computerized densitometry with the software package NIH Image 1.62 (developed at the US National Institutes of Health and available on-line at http://rsb.info.nih.gov/nih-image/). For autoradiography, optical grey densities were transformed into nCi/mg of tissue equivalent using a standard curve generated with tritium standards and then converted into fmol/mg of tissue using the specific activity of the radioligand. For in situ hybridization data, a standard transmission density scale (Stouffer Graphic Arts Equipments Inc., South Bend, IN, USA) was used, and the results were expressed as arbitrary optical density units. Non-specific signal, as assessed with excess of unlabelled probe, was subtracted from these values.

For analysis, caudate nucleus and putamen were divided in two subregions along the medial-lateral axis, whereas external segments of the globus pallidus (GPe) were divided along the dorso-ventral axis (see Fig. 2C). These subdivisions were based on the somatotopic representation of corticostriatal projections to the striatopallidal complex (see Fig. 2D) (Yoshida et al., 1993; Parent and Hazrati, 1995). Data were computed separately for each subregion and were grouped when regional effects were similar. Statistical comparisons of data were performed using an analysis of variance (ANOVA) followed by post hoc pairwise comparisons with Fisher’s probability of least significant difference test. \( P < 0.05 \) was considered significant. At first, the nine control subjects were compared with the 14 parkinsonian subjects. In subsequent analysis, comparisons were made between controls, parkinsonian non-dyskinetic and parkinsonian dyskinetic subjects. 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, Parkinson’s disease patients, and then Parkinson’s disease patients with controls altogether.

**Results**

Table 1 shows an extensive decrease in dopamine concentrations (−98.8%) and [125I]RTI-121-specific binding (−92%) in the putamen of Parkinson’s disease patients (for more details see Calon et al., 2003). Clinical data, markers of dopaminergic denervation and brain pH were similar between dyskinetic and non-dyskinetic levodopa-treated Parkinson’s disease patients (Table 1). However, parkinsonian subjects were older than controls. Correlation between age of death and A2AR mRNA or [3H]SCH 58261-specific binding to A2AR were tested, and no significant correlation was found in either controls or Parkinson’s disease patients (data not shown). No significant correlation was found between [3H]SCH 58261-specific binding and A2AR mRNA levels and other clinical variables such as development of wearing-off, duration of disease, duration of levodopa therapy, delay to autopsy, age of Parkinson’s disease onset, cumulative levodopa dose, duration of follow-up, age at levodopa initiation, duration of Parkinson’s disease at the initiation of levodopa, average daily dose of levodopa, brain pH or time delay in the freezer (data not shown).

Figures 1A and 2A show increased A2AR mRNA levels in the putamen (lateral and medial) of dyskinetic patients in comparison with controls (medial: mean difference = +0.20, critical difference = 0.017, \( P = 0.0191 \); lateral: mean difference = +0.033, critical difference = 0.020, \( P = 0.0022 \)). This increase of A2AR mRNA labelling in the lateral putamen was significant in dyskinetic patients compared with non-dyskinetic patients (mean difference = 0.0211, critical difference = 0.0208, \( P = 0.0470 \) (Figs 1A and 2A). No alterations of A2AR mRNA levels were seen in the caudate of dyskinetic Parkinson’s disease patients. Comparison between controls and Parkinson’s disease patients pooled in one group (\( n = 14 \)) showed an increase of A2AR mRNA levels in the lateral putamen in Parkinson’s disease patients (mean difference = +0.023, critical difference = 0.018, \( P = 0.0154 \), but not elsewhere in the caudate/putamen (data not shown). 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). Present data were compared with a previous in situ hybridization study of PPE expression in the same patients (Calon et al., 2002), and a significant positive correlation between A2AR and PPE mRNA expression levels was found in the caudate nucleus\( (n = 23, r^2 = 0.28, P < 0.01) \) and in the putamen\( (n = 23, r^2 = 0.46, P < 0.001) \) (data not shown).
[3H]SCH 58261-specific binding to A2AR was also increased in both lateral and medial putamen of dyskinetic patients compared with controls (medial: mean difference = +45.98, critical difference = 28.37, \( P = 0.0030 \); lateral: mean difference = +47.76, critical difference = 29.78, \( P = 0.0032 \)) (Figs 1B and 2B), but was not significantly different between dyskinetic and non-dyskinetic Parkinson’s disease patients (medial: mean difference = +25.60, critical difference = 30.09, \( P = 0.0912 \); lateral: mean difference = +20.277, critical difference = 31.587, \( P = 0.1956 \)). In addition, as shown in Figs 1B and 2B, [3H]SCH 58261-specific binding to A2AR was also increased in the ventral and dorsal GPe of Parkinson’s disease patients (dyskinetic and non-dyskinetic) compared with control subjects (Parkinson’s disease versus controls comparison, ventral: mean difference = +41.27, critical difference = 24.42, \( P = 0.0021 \); dorsal: mean difference = +39.81, critical difference = 19.94, \( P = 0.0005 \)). In contrast, no changes in [3H]SCH 58261 binding sites were observed in the caudate of dyskinetic Parkinson’s disease patients (Figs 1 and 2). Other statistical analyses show that the increase in [3H]SCH 58261-specific binding is also present in the putamen when all Parkinson’s disease patients pooled together are compared with controls (medial: mean difference = +33.18, critical difference = 25.18, \( P = 0.0123 \); lateral: mean difference = +37.63, critical difference = 25.64, \( P = 0.0061 \)) (data not shown). Grouping Parkinson’s disease patients revealed a significant decrease of [3H]SCH 58261-specific binding in the medial caudate compared with controls.

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**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 (A) A2AR mRNA and (B) [3H]SCH 58261 binding to A2AR in a control subject and in levodopa-treated parkinsonian patients with or without dyskinesias. CD = caudate; GPe = external segment of the globus pallidus; Put = putamen; A2AR = adenosine A2A receptor; PD = Parkinson disease.
No significant correlations were found between A2AR mRNA levels or [3H]SCH 58261-specific binding and markers of dopaminergic denervation (dopamine content or [125I]RTI-121-specific binding) in Parkinson's disease patients ($n = 14$). A2AR mRNA level and [3H]SCH 58261-specific binding in the internal segment of the globus pallidus were not significantly different from background.

Discussion

The dyskinetic patients included in this study suffered from Parkinson’s disease for an extended time (mean duration 15.4 $\pm$ 2.4 years), and received a prolonged therapy with levodopa (mean duration 10.7 $\pm$ 1.8 years), which was stopped on average a few days before the time of death. The fact that the present increase of A2AR mRNA level and [3H]SCH 58261-specific binding to A2AR in the putamen of dyskinetic patients was observed post mortem after years of treatment indicates that it is a long-lasting pathological adaptive alteration, and not a transient phenomenon. It is consistent with the hypothesis that chronic treatment with levodopa induces persistent changes in the brain, and with the observation that LID are generally considered poorly reversible in standard clinical practice (Calon et al., 2000; Rascol and Fabre, 2001). Moreover, our data showed no link between A2AR levels and other variables such as normal ageing, duration of Parkinson’s disease, daily dose of levodopa or duration of levodopa treatment. Increased A2AR was, rather, selectively linked to the occurrence of dyskinesias.

Previous studies in human have aimed to define the general distribution of A2AR mRNA (Schiffmann et al., 1991; Peterfreund et al., 1996; Svenningsson et al., 1998) and the A2AR binding sites using selective radioligands (Martinez-Mir et al., 1991; Svenningsson et al., 1997). Our data show a distribution pattern consistent with these reports, with a selective distribution of A2AR mRNA in the caudate/putamen and a localization of [3H]SCH 58261 binding...
mostly confined to the caudate/putamen and the GPe (Figs 1 and 2).

Other analyses have focused on the effect of dopaminergic lesions on A2AR. In situ hybridization studies in rat brain sections show either no changes or a 20% increase of A2AR gene expression in the striatum after lesion of the dopaminergic pathways with 6-OHDA (Kaelin-Lang et al., 2000; Pinna et al., 2002). A previous reverse transcription polymerase chain reaction (RT–PCR) study compared the levels of A2AR mRNA in some regions of the caudate/putamen of Parkinson’s disease patients with matched controls (Hurley et al., 2000). A significant decrease in the level of A2AR mRNA was described in the anterior and posterior caudate nucleus and anterior dorsal putamen in Parkinsonian brain compared with controls. However, no change was seen in the ventral posterior part of the putamen in parkinsonian disease patients, a region that corresponds approximately to the putaminal areas studied in the present work. Overall, these data are slightly different from the present results, as we show an increase in A2AR mRNA transcripts in the posterior putamen in Parkinson's disease patients, and that the increase is more prominent in Parkinson’s disease patients with LID. Although it is known that the patients from that previous analysis received levodopa, data on LID were not available. Therefore, methodological difference (i.e. regular RT–PCR versus in situ hybridization) and difference in the motor complication profile of patients may explain these discrepancies. On the other hand, consistent with our data, Zeng et al. (2000) previously found a link between increased A2AR mRNA expression in the putamen and the development of dyskinesias in normal monkeys following a high dose of levodopa.

A previous study showed no change of [3H]CGS 21680 binding sites to A2AR in the striatum of guinea pig with chronic 6-OHDA lesion of dopaminergic system (Martinez-Mir et al., 1991). Accordingly, no alteration of [3H]CGS 21680 binding to A2AR was observed in the striatum and pallidum of Parkinson’s disease patients compared with controls (Martinez-Mir et al., 1991). However, the number of Parkinson’s disease patients included in the study was limited (n = 3–7), and [3H]CGS 21680 is a A2AR agonist that has been shown to be less reliable than SCH 58261 for quantitative studies of A2AR (Cunha et al., 1996; Lindstrom et al., 1996; Fredholm et al., 1998). Moreover, the relation with dyskinesia and [3H]CGS 21680 was not studied directly. These factors may account for the differences from our present results.

The changes in A2AR mRNA and [3H]SCH 58261-specific binding to A2AR in the present analysis were predominantly observed in the putamen, and more specifically in the lateral putamen. Studies in non-human primate at a rostro-caudal level comparable to our report demonstrate that inputs from sensorimotor areas innervate mainly the putamen and its lateral parts, whereas inputs from associative cortical areas terminate in caudate nucleus (Kemp and Powell, 1970; Parent, 1990; Parent and Hazrati, 1995). This topographic organization of cortical projections in the striatum is depicted in Fig. 2D. Furthermore, compared with the rest of the striatum, the lateral putamen is associated with the larger dyskinesia-related increase in PPE mRNA (Morissette et al., 1997; Calon et al., 2002), and with the more prominent loss of [125I]RTI-121-specific binding to dopamine transporter in the same Parkinson’s disease patients (Calon et al., 2003). The fact that these changes are restricted to a striatal region involved in motor behaviour strengthens the hypothesis that A2AR alterations are linked to the pathophysiology of a motor disorder such as LID.

As described in the Introduction, A2AR antagonist administration in MPTP monkeys was reported to improve the antiparkinsonian effect and the dyskinesigenic profile of levodopa (Grondin et al., 1999; Kanda et al., 2000). Moreover, data gathered from rodent models of levodopa-induced motor complications and in dyskinetic monkeys suggest a role of A2AR in these adverse effects of levodopa (Zeng et al., 2000; Morelli and Pinna, 2001; Pinna et al., 2001; Bove et al., 2002; Freduzzi et al., 2002; Bibbiani et al., 2003; Lundblad et al., 2003). As our results show an abnormal expression of A2AR in dyskinetic levodopa-treated Parkinson’s disease patients, it is possible that A2AR antagonist treatment works against the pathophysiological effect of this increase. This action might contribute to the mechanism of action of the antidyskinesigenic effect of A2AR antagonists.

It has been shown that A2AR activation regulates PPE mRNA expression in rat striatopallidal neurons, and that this regulatory role is strongly influenced by dopamine depletion (Svenningsson et al., 1999). For instance, administration of A2AR antagonist reverses the PPE mRNA increase induced in the rat striatum by dopaminergic lesions alone or the combination of dopaminergic lesions and subsequent dopaminergic treatment (Schiffmann and Vanderhaeghen, 1993; Aoyama et al., 2002; Carta et al., 2002; Lundblad et al., 2003). Genetic inactivation of A2AR also partially blocks the increase in PPE expression in the striatum caused by D2 dopamine receptor deficiency or haloperidol treatment (Chen et al., 2001). Association between increased expression of PPE and the development of LID has been well documented in MPTP monkeys (Morissette et al., 1997; Calon et al., 2000; Zeng et al., 2000). Although a causal link between enkephalin and LID has not been proven, dopaminomimetic treatments that induce dyskinesias in MPTP monkey consistently fail to correct the increased PPE expression induced by dopaminergic depletion (Calon et al., 2000; Quik et al., 2002). In a previous analysis in the same patients, increased PPE mRNA expression was also found in the lateral putamen of dyskinetic subjects (Calon et al., 2002). Our results further show that the levels of A2AR mRNA correlate positively with those of PPE in the putamen of these same Parkinson’s disease patients.

This leads to the intriguing possibility that A2AR elevation might be a pathological event that precedes increased PPE expression. Indeed, increased numbers of A2AR in striatopallidal cells may enhance their sensitivity to adenosine.
stimulation and contribute to a chronic state of PPE overexpression. Therefore, the mechanism of action of A2AR antagonists in the treatment of LID may involve a rectification of the increase of PPE gene expression. Further studies on the relationship between the antidysonetic action of A2AR antagonist and the expression of PPE in animal models of LID are needed to address this issue. Moreover, this interpretation relies on the assumption that increased PPE expression plays a causal role in LID, which remains to be established.

According to the present data, patients experiencing LID have increased A2AR mRNA and, to a lesser extent, increased [3H]SCH 58261, in their putamen. This suggests that a chronic acceleration in the synthesis of the A2AR is present in the striatopallidal projection of these patients. This increase of mRNA was partly translated at the level of the protein, consistent with the view that A2AR undergoes important post-transcriptional regulation in vitro (Lee et al., 1999). Transcriptional regulation of the A2AR gene is a potential mechanism, since sequences known to bind the transcription factors NF-xB, activator protein-1 (AP-1) and AP-2 have been identified in the 5’ flanking sequence of the gene (Chu et al., 1996). Interestingly, the promoter sequences of PPE and other important genes expressed in striatal neurons, including NR2B, NR1 and prodynorphin, contain an AP-1 consensus site (Bai and Kusiak, 1993; Cole et al., 1995; Weisinger, 1995; Klein et al., 1998). Hence, both A2AR and PPE gene expression in LID may be amplified by a common transcription factor. For example, FosB-related transcription factors interact with Jun-D to form an AP-1 dimer (Vallone et al., 1997; Andersson et al., 2001) that could in theory regulate A2AR and PPE mRNA transcription. ∆FosB-related proteins have been suggested to play a important role in the pathogenesis of LID (Calon et al., 2000), based on series of studies in 6-OHDA rats (Cenci, 2002) and on the fact that ∆FosB is increased in the striatum of dyskinetic MPTP monkeys (Doucet et al., 1996). To test this hypothesis, ∆FosB immunoreactivity was measured in the putamen of the patients included in the present study, and was found to be increased in Parkinson’s disease patients, without differentiating the dyskinetic from the non-dyskinetic Parkinson’s disease patients (Tekumalla et al., 2001). FosB levels did not correlate with A2AR mRNA levels or [3H]SCH 58261 binding sites (data not shown). Although FosB-related transcription factors may still play a role in short-term generation of dyskinesias, other unknown transcription factors may still play a role in short-term generation of dyskinesias, other unknown transcription factors may still play a role in chronic upregulation of A2AR seen in the present study after sustained levodopa therapy.

It is common sense to think that increased A2AR expression in neurons of the striatopallidal pathway would modulate their activity. Recent indications of a close association between A2AR and D2 dopamine receptor also suggest that an alteration in A2AR would affect the motor response to levodopa treatment (Ferre et al., 2001; Hillion et al., 2002). However, it is difficult to predict the exact motor consequences on LID of such change based on existing functional anatomy models of basal ganglia in LID (Albin et al., 1989; Blanchet et al., 1994; Crossman, 2000; Obeso et al., 2000b; Yelnik et al., 2000). Indeed, there is no consensus on whether it is an increase or a decrease of the striatopallidal activity that is causally linked with LID. Therefore, our data reveal an important pathological link between A2AR and LID, but the exact mechanism underlying this link remains unknown.

**Conclusions**

The results of this study are consistent with the following conclusions. First, the development of dyskinesias is associated with post mortem increased levels of A2AR mRNA and [3H]SCH 58261-specific binding to A2AR in the putamen of human Parkinson’s disease patients. Secondly, A2AR mRNA in the lateral putamen in dyskinetic Parkinson’s disease patients is higher than in non-dyskinetic patients. Thirdly, [3H]SCH 58261-specific binding to A2AR is increased in the GPe of Parkinson’s disease patients, regardless of the development of motor complications to levodopa. These observations suggest that putaminal A2AR overexpression is persistently involved in the pathogenesis of LID and Parkinson’s disease in human. These adaptive changes may be causally linked to LID per se or through regulation of PPE expression, but may also be a consequence of the dyskinetic process. Overall, our data substantiate the notion that A2AR antagonists may be useful in the treatment of Parkinson’s disease and LID.

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