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Increased adenosine A_{2A} receptors in the brain of Parkinson's disease patients with dyskinesias

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Summary

Brain adenosine A_{2A} 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 A_{2A} receptor mRNA and [^{3}H]SCH 58261-specific binding to adenosine A_{2A} 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 [^{125}I]RTI-121-specific binding to dopamine transporters. A_{2A} receptor mRNA levels (+129%; $P < 0.01$) and [^{3}H]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 A_{2A} 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, [^{3}H]SCH 58261-specific binding to adenosine A_{2A} 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 A_{2A} receptors was observed in the caudate nucleus, whereas adenosine A_{2A} receptor protein and mRNA levels in the internal globus pallidus were not different from background. These findings suggest that increased synthesis of adenosine A_{2A} receptors in striatopallidal pathway neurons is associated with the development of dyskinesias following long-term levodopa therapy in Parkinson's disease.

Keywords: adenosine A_{2A} receptors; motor complications; Parkinson's disease; putamen; SCH 58261

Abbreviations: AP = activator protein; A2AR = adenosine A_{2A} 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

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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*., 2000*a*; 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 dyskinesigenic profile of levodopa alone. In this regard, adenosine A_{2A} 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; Schiffmann *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*., 2000*b*; 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 behavioural 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, behavioural 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 dyskinesia 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 [^{125}I]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

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

	n	Age at death (years)	Delay to autopsy (h)	Brain tissue (pH)	Age of PD onset (years)	Duration of PD (years)	Duration of levodopa use (years)	Cumulative levodopa dose (g)	Putamen	
									[^{125}I]RTI-121-specific binding (amol/mg tissue)	Dopamine concentration (ng/mg protein)
Controls	9	68 ± 3	<24	6.37 ± 0.07	–	–	–	–	830 ± 68	63.87 ± 4.89
PD	14	78 ± 2*	12 ± 2	6.37 ± 0.03	62 ± 4	16.2 ± 2.1	11.2 ± 1.6	13 651 ± 3413	69 ± 6**	0.77 ± 0.14**
PD, LID	7	80 ± 3	14 ± 2	6.36 ± 0.05	63 ± 3	17.4 ± 3.5	11.9 ± 2.8	15 316 ± 6422	70 ± 10	0.72 ± 0.22
PD, no LID	7	77 ± 2	11 ± 3	6.39 ± 0.04	61 ± 2	15.4 ± 2.4	10.7 ± 1.8	11 984 ± 2884	69 ± 9	0.82 ± 0.17

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

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 of levodopa-only (without decarboxylase) formulation according to Markham and Diamond (1981). Some patients were receiving other antiparkinsonian drugs, such as a dopamine agonist (bromocriptine), amantadine, anticholinergic drugs or selegiline, but the use of these alternate drugs was similar in the dyskinetic and the non-dyskinetic groups of patients (Calon *et al*., 2003). Although no records of theophylline treatment or high caffeine consumption was found in our patient files, the effect of these common adenosine receptor modulators on A2AR expression cannot be ruled out, and could be a confounding variable in the present study.

Autopsy and handling of the brain material

The brains of the 14 Parkinson's disease patients were obtained, as well as those from nine controls (including one from the Douglas Hospital Research Center brain bank, Montreal, Canada) who died with no neurological disorders. All autopsies were carried out within 24 h of death. One half of the brain was immediately frozen at –80°C. The other half of the brain was fixed in formalin and histologically examined. Only those individuals who had Lewy body Parkinson's disease were included in this report. The frozen half of the brain was cut by hand in the frontal plane into 2- to 3-mm thick slices. Slices containing the basal ganglia portion from the patients and controls were provided blindly to the analysts.

Biochemistry

Small punches (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.

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 [^{125}I]RTI-121 [3β-(4-^{125}I-iodophenyl)tropane-2β-carboxylic acid isopropyl ester] (2200 Ci/mmol; 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 [^{35}S]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 ± 10^6 cpm/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 denaturated 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, 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).

[^{3}H]SCH 58261 binding autoradiography

A2AR were evaluated using a high-affinity selective antagonist, [^{3}H]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 [^{3}H]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′-*N*-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 ^{3}H-sensitive films (Hyperfilm; Amersham, Baie d'Urfé, Quebec, Canada) along with tritium standards (^{3}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 subregions 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 [^{125}I]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 [^{3}H]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 [^{3}H]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).

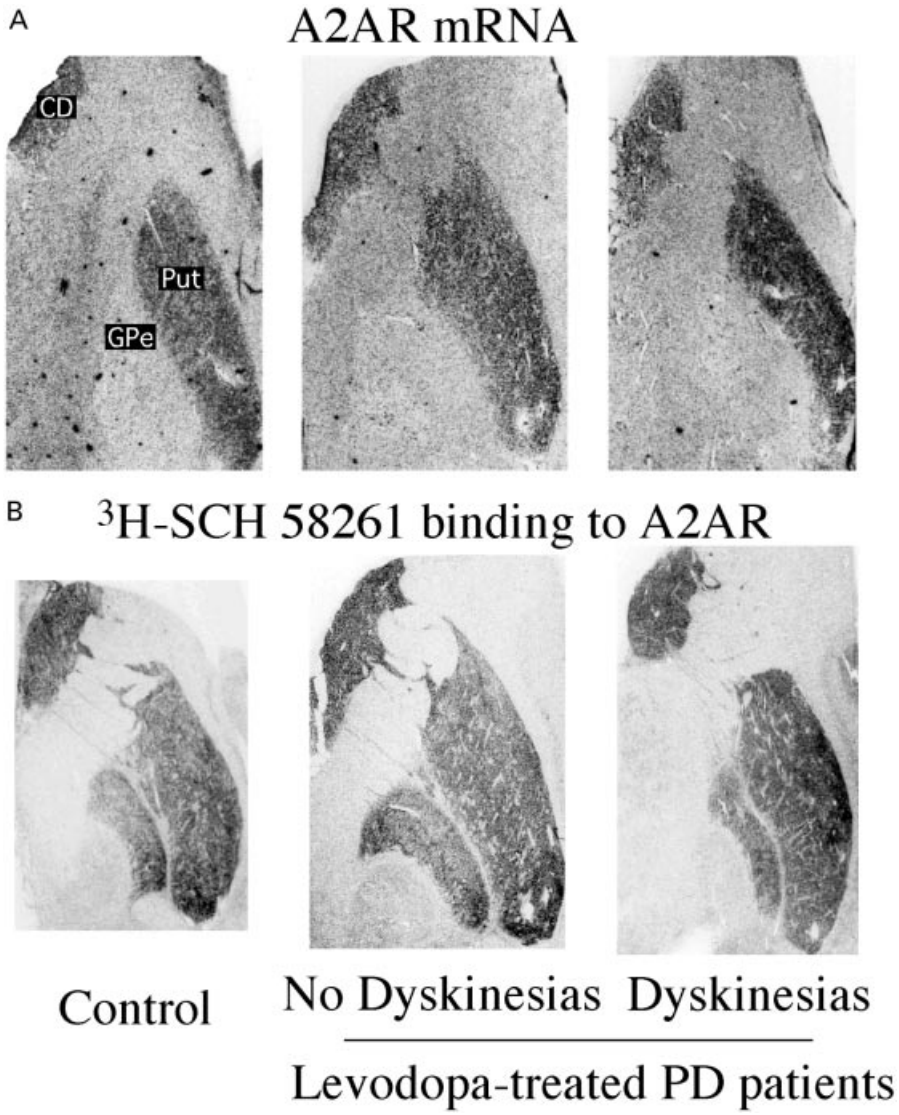

Fig. 1 Representative autoradiograms of human brain sections at the level of the caudate, putamen, and external and internal segments of the globus pallidus, showing (**A**) A2AR mRNA and (**B**) [^{3}H]SCH 58261 binding to A2AR in a control subject and in levodopa-treated parkinsonian patients with or without dyskinesias. CD = caudate; GPe = external segment of the globus pallidus; Put = putamen; A2AR =adenosine A_{2A} receptor; PD = Parkinson disease.

[^{3}H]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, [^{3}H]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 [^{3}H]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 [^{3}H]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 [^{3}H]SCH 58261-specific binding in the medial caudate compared with controls

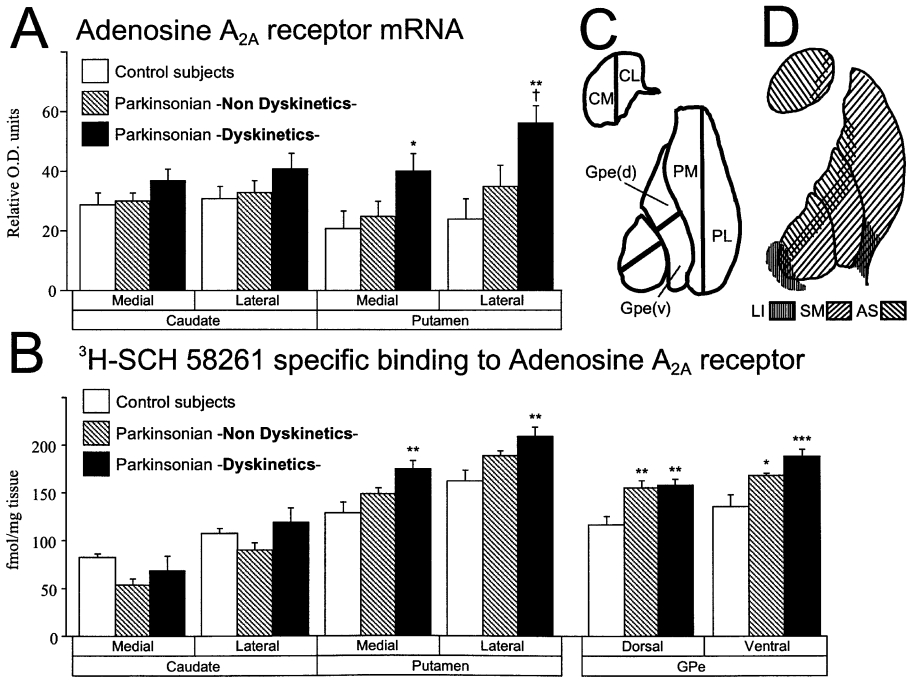

Fig. 2 (**A**) A2AR mRNA expression in the caudate nucleus and the putamen of *post mortem* human tissue from control and parkinsonian subjects in relation to the development of dyskinesias following levodopa therapy. Values are mean ± SEM, expressed in relative OD units. **P* < 0.05 and ***P* < 0.01 versus control subjects; †*P* < 0.05 versus Parkinsonian patients without dyskinesias using an ANOVA followed by *post hoc* pairwise comparisons with Fisher's probability of least significant difference test. (**B**) [^{3}H]SCH 58261-specific binding to A2AR in the caudate nucleus, the putamen and the GPe of *post mortem* human tissue from control and parkinsonian subjects in relation to the development of dyskinesias following levodopa therapy. Values shown are expressed in fmol/mg of tissue as the mean ± SEM. **P* < 0.05, ***P* < 0.01 and ****P* < 0.005 versus control subjects. (**C**) Actual division of the caudate nucleus, putamen and GPe used for quantitation of autoradiograms. GPe = external segment of the globus pallidus; Put = putamen; L = lateral; M = medial; v = ventral; d = dorsal. (**D**) Schematic representation of the caudate, putamen, and external and internal segments of the globus pallidus showing localization of associative (AS), sensorimotor (SM) and limbic (LI) striatal territories in primates based on corticostriatal projections (adapted from Parent and Hazrati, 1995).

(mean difference = –22.09, critical difference = 21.68, $P = 0.0462$) (data not shown). No significant correlations were found between A2AR mRNA levels or [^{3}H]SCH 58261-specific binding and markers of dopaminergic denervation (dopamine content or [^{125}I]RTI-121-specific binding) in Parkinson's disease patients ($n = 14$). A2AR mRNA level and [^{3}H]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 ± 2.4 years), and received a prolonged therapy with levodopa (mean duration 10.7 ± 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 [^{3}H]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 [^{3}H]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 parkinson's 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 [^{3}H]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 [^{3}H]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 [^{3}H]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 [^{3}H]CGS 21680 was not studied directly. These factors may account for the differences from our present results.

The changes in A2AR mRNA and [^{3}H]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 [^{125}I]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; Fredduzzi *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 antidyskinetic 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 [^{3}H]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-κB, 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. ΔFosB-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 ΔFosB is increased in the striatum of dyskinetic MPTP monkeys (Doucet *et al*., 1996). To test this hypothesis, ΔFosB 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 [^{3}H]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 be more likely to be involved in the 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*., 2000*b*; 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 [^{3}H]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, [^{3}H]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 adaptative 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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References

Ahlskog JE, Muenter MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Mov Disord 2001; 16: 448–58.

Albin RL, Young AB, Penney JB. The functional anatomy of basal ganglia disorders. Trends Neurosci 1989; 12: 366–75.

Andersson M, Konradi C, Cenci MA. cAMP response element-binding protein is required for dopamine-dependent gene expression in the intact but not the dopamine-denervated striatum. J Neurosci 2001; 21: 9930–43.

Aoyama S, Koga K, Mori A, Miyaji H, Sekine S, Kase H, et al. Distribution of adenosine A(2A) receptor antagonist KW-6002 and its effect on gene expression in the rat brain. Brain Res 2002; 953: 119–25.

Aubert I, Ghorayeb I, Normand E, Bloch B. Phenotypical characterization of

the neurons expressing the D1 and D2 dopamine receptors in the monkey striatum. J Comp Neurol 2000; 418: 22–32.

Bai G, Kusiak JW. Cloning and analysis of the 5′ flanking sequence of the rat N-methyl-D-aspartate receptor 1 (NMDAR1) gene. Biochim Biophys Acta 1993; 1152: 197–200.

Bara-Jimenez W, Sherzai A, Dimitrova T, Favit A, Bibbiani F, Gillespie M, et al. Adenosine A(2A) receptor antagonist treatment of Parkinson's disease. Neurology 2003; 61: 293–6.

Bibbiani F, Oh JD, Petzer JP, Castagnoli N, Chen JF, Schwarzschild MA, et al. A(2A) antagonist prevents dopamine agonist-induced motor complications in animal models of Parkinson's disease. Exp Neurol 2003; 184: 285–94.

Blanchet PJ, Boucher R, Bedard PJ. Excitotoxic lateral pallidotomy does not relieve L-dopa-induced dyskinesia in MPTP parkinsonian monkeys. Brain Res 1994; 650: 32–9.

Blanchet PJ, Allard P, Gregoire L, Tardif F, Bedard PJ. Risk factors for peak dose dyskinesia in 100 levodopa-treated parkinsonian patients. Can J Neurol Sci 1996; 23: 189–93.

Blanchet PJ, Konitsiotis S, Whittemore ER, Zhou ZL, Woodward RM, Chase TN. Differing effects of N-methyl-D-aspartate receptor subtype selective antagonists on dyskinesias in levodopa-treated 1-methyl-4-phenyl-tetrahydropyridine monkeys. J Pharmacol Exp Ther 1999; 290: 1034–40.

Bove J, Marin C, Bonastre M, Tolosa E. Adenosine A2A antagonism reverses levodopa-induced motor alterations in hemiparkinsonian rats. Synapse 2002; 46: 251–7.

Brotchie JM. Adjuncts to dopamine replacement: a pragmatic approach to reducing the problem of dyskinesia in Parkinson's disease. Mov Disord 1998; 13: 871–6.

Brotchie JM. CB(1) cannabinoid receptor signalling in Parkinson's disease. Curr Opin Pharmacol 2003; 3: 54–61.

Calon F, Di Paolo T. Levodopa response motor complications—GABA receptors and preproenkephalin expression in human brain. Parkinsonism Relat Disord 2002; 8: 449–54.

Calon F, Tahar AH, Blanchet PJ, Morissette M, Grondin R, Goulet M, et al. Dopamine-receptor stimulation: biobehavioral and biochemical consequences. Trends Neurosci 2000; 23 (10 Suppl): S92–100.

Calon F, Birdi S, Rajput AH, Hornykiewicz O, Bedard PJ, Di Paolo T. Increase of preproenkephalin mRNA levels in the putamen of Parkinson disease patients with levodopa-induced dyskinesias. J Neuropathol Exp Neurol 2002; 61: 186–96.

Calon F, Morissette M, Rajput AH, Hornykiewicz O, Bedard PJ, Di Paolo T. Changes of GABA receptors and dopamine turnover in the postmortem brains of parkinsonians with levodopa-induced motor complications. Mov Disord 2003; 18: 241–53.

Carta AR, Pinna A, Cauli O, Morelli M. Differential regulation of GAD67, enkephalin and dynorphin mRNAs by chronic-intermittent L-dopa and A2A receptor blockade plus L-dopa in dopamine-denervated rats. Synapse 2002; 44: 166–74.

Cenci MA. Transcription factors involved in the pathogenesis of L-DOPA-induced dyskinesia in a rat model of Parkinson's disease. Amino Acids 2002; 23: 105–9.

Chen JF, Moratalla R, Impagnatiello F, Grandy DK, Cuellar B, Rubinstein M, et al. The role of the D(2) dopamine receptor (D(2)R) in A(2A) adenosine receptor (A(2A)R)-mediated behavioral and cellular responses as revealed by A(2A) and D(2) receptor knockout mice. Proc Natl Acad Sci USA 2001; 98: 1970–5.

Chu YY, Tu KH, Lee YC, Kuo ZJ, Lai HL, Chern Y. Characterization of the rat A2a adenosine receptor gene. DNA Cell Biol 1996; 15: 329–37.

Cole RL, Konradi C, Douglass J, Hyman SE. Neuronal adaptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. Neuron 1995; 14: 813–23.

Crossman AR. Functional anatomy of movement disorders. J Anat 2000; 196: 519–25.

Cunha RA, Johansson B, Constantino MD, Sebastiao AM, Fredholm BB. Evidence for high-affinity binding sites for the adenosine A2A receptor agonist [3H] CGS 21680 in the rat hippocampus and cerebral cortex that are different from striatal A2A receptors. Naunyn Schmiedebergs Arch Pharmacol 1996; 353: 261–71.

Dionisotti S, Ongini E, Zocchi C, Kull B, Arslan G, Fredholm BB. Characterization of human A2A adenosine receptors with the antagonist radioligand [3H]-SCH 58261. Br J Pharmacol 1997; 121: 353–60.

Doucet JP, Nakabeppu Y, Bedard PJ, Hope BT, Nestler EJ, Jasmin BJ, et al. Chronic alterations in dopaminergic neurotransmission produce a persistent elevation of deltaFosB-like protein(s) in both the rodent and primate striatum. Eur J Neurosci 1996; 8: 365–81.

Fahn S. The spectrum of levodopa-induced dyskinesias. Ann Neurol 2000; 47 (4 Suppl 1): S2–9.

Ferre S, Popoli P, Gimenez-Llort L, Rimondini R, Muller CE, Stromberg I, et al. Adenosine/dopamine interaction: implications for the treatment of Parkinson's disease. Parkinsonism Relat Disord 2001; 7: 235–41.

Fredduzzi S, Moratalla R, Monopoli A, Cuellar B, Xu K, Ongini E, et al. Persistent behavioral sensitization to chronic L-DOPA requires A2A adenosine receptors. J Neurosci 2002; 22: 1054–62.

Fredholm BB, Lindstrom K, Dionisotti S, Ongini E. [3H]SCH 58261, a selective adenosine A2A receptor antagonist, is a useful ligand in autoradiographic studies. J Neurochem 1998; 70: 1210–6.

Furlong TJ, Pierce KD, Selbie LA, Shine J. Molecular characterization of a human brain adenosine A2 receptor. Brain Res Mol Brain Res 1992; 15: 62–6.

Grondin R, Bedard PJ, Hadj Tahar A, Gregoire L, Mori A, Kase H. Antiparkinsonian effect of a new selective adenosine A2A receptor antagonist in MPTP-treated monkeys. Neurology 1999; 52: 1673–7.

Henry B, Fox SH, Crossman AR, Brotchie JM. Mu- and delta-opioid receptor antagonists reduce levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Exp Neurol 2001; 171: 139–46.

Hillion J, Canals M, Torvinen M, Casado V, Scott R, Terasmaa A, et al. Coaggregation, cointernalization, and codesensitization of adenosine A2A receptors and dopamine D2 receptors. J Biol Chem 2002; 277: 18091–7.

Hoehn MM, Yahr MD. Parkinsonism: onset, progression and mortality. Neurology 1967; 17: 427–42.

Hurley MJ, Mash DC, Jenner P. Adenosine A(2A) receptor mRNA expression in Parkinson's disease. Neurosci Lett 2000; 291: 54–8.

Jenner P. Factors influencing the onset and persistence of dyskinesia in MPTP-treated primates. Ann Neurol 2000; 47 (4 Suppl 1): S90–9.

Kaelin-Lang A, Liniger P, Probst A, Lauterburg T, Burgunder JM. Adenosine A2A receptor gene expression in the normal striatum and after 6-OH-dopamine lesion. J Neural Transm 2000; 107: 851–9.

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, et al. Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann Neurol 1998; 43: 507–13.

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, et al. Combined use of the adenosine A(2A) antagonist KW-6002 with L-DOPA or with selective D1 or D2 dopamine agonists increases antiparkinsonian activity but not dyskinesia in MPTP-treated monkeys. Exp Neurol 2000; 162: 321–7.

Kase H. New aspects of physiological and pathophysiological functions of adenosine A2A receptor in basal ganglia. Biosci Biotechnol Biochem 2001; 65: 1447–57.

Kemp JM, Powell TP. The cortico-striate projection in the monkey. Brain 1970; 93: 525–46.

Kingsbury AE, Foster OJ, Nisbet AP, Cairns N, Bray L, Eve DJ, et al. Tissue pH as an indicator of mRNA preservation in human post-mortem brain. Brain Res Mol Brain Res 1995; 28: 311–8.

Klein M, Pieri I, Uhlmann F, Pfizenmaier K, Eisel U. Cloning and characterization of promoter and 5′-UTR of the NMDA receptor subunit epsilon 2: evidence for alternative splicing of 5′-non-coding exon. Gene 1998; 208: 259–69.

Kostic VS, Svetel M, Sternic N, Dragasevic N, Przedborski S. Theophylline increases 'on' time in advanced parkinsonian patients. Neurology 1999; 52: 1916.

Kulisevsky J, Barbanoj M, Gironell A, Antonijoan R, Casas M, Pascual-

Sedano B. A double-blind crossover, placebo-controlled study of the adenosine A2A antagonist theophylline in Parkinson's disease. Clin Neuropharmacol 2002; 25: 25–31.

Lee YC, Chang CW, Su CW, Lin TN, Sun SH, Lai HL, et al. The 5′ untranslated regions of the rat A2A adenosine receptor gene function as negative translational regulators. J Neurochem 1999; 73: 1790–8.

Lindstrom K, Ongini E, Fredholm BB. The selective adenosine A2A receptor antagonist SCH 58261 discriminates between two different binding sites for [3H]-CGS 21680 in the rat brain. Naunyn Schmiedebergs Arch Pharmacol 1996; 354: 539–41.

Lundblad M, Vaudano E, Cenci MA. Cellular and behavioural effects of the adenosine A2a receptor antagonist KW-6002 in a rat model of l-DOPA-induced dyskinesia. J Neurochem 2003; 84: 1398–410.

Mally J, Stone TW. The effect of theophylline on parkinsonian symptoms. J Pharm Pharmacol 1994; 46: 515–7.

Markham CH, Diamond SG. Evidence to support early levodopa therapy in Parkinson disease. Neurology 1981; 31: 125–31.

Martinez-Mir MI, Probst A, Palacios JM. Adenosine A2 receptors: selective localization in the human basal ganglia and alterations with disease. Neuroscience 1991; 42: 697–706.

Miyawaki E, Lyons K, Pahwa R, Troster AI, Hubble J, Smith D, et al. Motor complications of chronic levodopa therapy in Parkinson's disease. Clin Neuropharmacol 1997; 20: 523–30.

Morelli M, Pinna A. Interaction between dopamine and adenosine A2A receptors as a basis for the treatment of Parkinson's disease. Neurol Sci 2001; 22: 71–2.

Morissette M, Goulet M, Soghomonian JJ, Blanchet PJ, Calon F, Bedard PJ, et al. Preproenkephalin mRNA expression in the caudate-putamen of MPTP monkeys after chronic treatment with the D2 agonist U91356A in continuous or intermittent mode of administration: comparison with L-DOPA therapy. Brain Res Mol Brain Res 1997; 49: 55–62.

Obeso JA, Olanow CW, Nutt JG. Levodopa motor complications in Parkinson's disease. Trends Neurosci 2000a; 23 (10 Suppl): S2–7.

Obeso JA, Rodriguez-Oroz MC, Rodriguez M, DeLong MR, Olanow CW. Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. Ann Neurol 2000b; 47 (4 Suppl 1): S22–32.

Parent A. Extrinsic connections of the basal ganglia. Trends Neurosci 1990; 13: 254–8.

Parent A, Hazrati LN. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Brain Res Rev 1995; 20: 91–127.

Peterfreund RA, MacCollin M, Gusella J, Fink JS. Characterization and expression of the human A2a adenosine receptor gene. J Neurochem 1996; 66: 362–8.

Pinna A, Fenu S, Morelli M. Motor stimulant effects of the adenosine A2A receptor antagonist SCH 58261 do not develop tolerance after repeated treatments in 6-hydroxydopamine-lesioned rats. Synapse 2001; 39: 233–8.

Pinna A, Corsi C, Carta AR, Valentini V, Pedata F, Morelli M. Modification of adenosine extracellular levels and adenosine A(2A) receptor mRNA by dopamine denervation. Eur J Pharmacol 2002; 446: 75–82.

Ponte P, Ng SY, Engel J, Gunning P, Kedes L. Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA. Nucleic Acids Res 1984; 12: 1687–96.

Quik M, Police S, Langston JW, Di Monte DA. Increases in striatal preproenkephalin gene expression are associated with nigrostriatal damage but not L-DOPA-induced dyskinesias in the squirrel monkey. Neuroscience 2002; 113: 213–20.

Rajput AH, Fenton ME, Birdi S, Macaulay R, George D, Rozdilsky B, et al. Clinical-pathological study of levodopa complications. Mov Disord 2002; 17: 289–96.

Rascol O, Fabre N. Dyskinesia: L-dopa-induced and tardive dyskinesia. Clin Neuropharmacol 2001; 24: 313–23.

Rascol O, Brooks DJ, Korczyn AD, De Deyn PP, Clarke CE, Lang AE. A five-year study of the incidence of dyskinesia in patients with early Parkinson's disease who were treated with ropinirole or levodopa. 056 Study Group. N Engl J Med 2000; 342: 1484–91.

Richardson PJ, Kase H, Jenner PG. Adenosine A2A receptor antagonists as new agents for the treatment of Parkinson's disease. Trends Pharmacol Sci 1997; 18: 338–44.

Schiffmann SN, Vanderhaeghen JJ. Adenosine A2 receptors regulate the gene expression of striatopallidal and striatonigral neurons. J Neurosci 1993; 13: 1080–7.

Schiffmann SN, Libert F, Vassart G, Vanderhaeghen JJ. Distribution of adenosine A2 receptor mRNA in the human brain. Neurosci Lett 1991; 130: 177–81.

Staley JK, Boja JW, Carroll FI, Seltzman HH, Wyrick CD, Lewin AH, et al. Mapping dopamine transporters in the human brain with novel selective cocaine analog [125I]RTI-121. Synapse 1995; 21: 364–72.

Svenningsson P, Hall H, Sedvall G, Fredholm BB. Distribution of adenosine receptors in the postmortem human brain: an extended autoradiographic study. Synapse 1997; 27: 322–35.

Svenningsson P, Le Moine C, Aubert I, Burbaud P, Fredholm BB, Bloch B. Cellular distribution of adenosine A2A receptor mRNA in the primate striatum. J Comp Neurol 1998; 399: 229–40.

Svenningsson P, Le Moine C, Fisone G, Fredholm BB. Distribution, biochemistry and function of striatal adenosine A2A receptors. Prog Neurobiol 1999; 59: 355–96.

Tekumalla PK, Calon F, Rahman Z, Birdi S, Rajput AH, Hornykiewicz O, et al. Elevated levels of DeltaFosB and RGS9 in striatum in Parkinson's disease. Biol Psychiatry 2001; 50: 813–6.

Vallone D, Pellecchia MT, Morelli M, Verde P, DiChiara G, Barone P. Behavioural sensitization in 6-hydroxydopamine-lesioned rats is related to compositional changes of the AP-1 transcription factor: evidence for induction of FosB- and JunD-related proteins. Brain Res Mol Brain Res 1997; 52: 307–17.

Weisinger G. The transcriptional regulation of the preproenkephalin gene. Biochem J 1995; 307: 617–29.

Yelnik J, Damier P, Bejjani BP, Francois C, Gervais D, Dormont D, et al. Functional mapping of the human globus pallidus: contrasting effect of stimulation in the internal and external pallidum in Parkinson's disease. Neuroscience 2000; 101: 77–87.

Yoshida S, Nambu A, Jinnai K. The distribution of the globus pallidus neurons with input from various cortical areas in the monkeys. Brain Res 1993; 611: 170–4.

Zeng BY, Pearce RK, MacKenzie GM, Jenner P. Alterations in preproenkephalin and adenosine-2a receptor mRNA, but not preprotachykinin mRNA correlate with occurrence of dyskinesia in normal monkeys chronically treated with L-DOPA. Eur J Neurosci 2000; 12: 1096–104.