Upregulation of dopamine D₃, not D₂, receptors correlates with tardive dyskinesia in a primate model

Souha Mahmoudi, PhD¹, Daniel Lévesque, PhD^{1,3}, and Pierre J. Blanchet, MD, FRCPC, PhD^{2,3,*}

¹Faculty of Pharmacy, Université de Montréal, Montréal, Québec, Canada

²Faculty of Dental Medicine, Department of Stomatology, Université de Montréal, Canada

³CNS Research Group (GRSNC), Université de Montréal, Canada

Abstract

Background—Tardive dyskinesia (TD) is a delayed and potentially irreversible motor complication arising in patients chronically exposed to centrally-active dopamine D₂ receptor antagonists, including antipsychotic drugs and metoclopramide. The classical dopamine D₂ receptor supersensitivity hypothesis in TD, which stemmed from rodent studies, lacks strong support in humans.

Methods—To investigate the neurochemical basis of TD, we chronically exposed adult capuchin monkeys to haloperidol (median 18.5 months, N=11) or clozapine (median 6 months, N=6). Six unmedicated animals were used as controls. Five haloperidol-treated animals developed mild TD movements, and no TD was observed in the clozapine group. Using receptor autoradiography, we measured dopamine D₁, D₂, and D₃ receptor levels. We also examined the D₃ receptor/preprotachykinin mRNA co-expression, and quantified preproenkephalin mRNA levels, in striatal sections.

Results—Unlike clozapine, haloperidol strongly induced dopamine D₃ receptor binding sites in the anterior caudate-putamen, particularly in TD animals, and binding levels positively correlated with TD intensity. Interestingly, D₃ receptor upregulation is observed in striatonigral output pathway. In contrast, D₂ receptor binding was comparable to controls, and dopamine D₁ receptor binding was reduced in the anterior (haloperidol and clozapine) and posterior (clozapine) putamen. Enkephalin mRNA widely increased in all animals, but to a greater extent in TD-free animals.

Conclusions—These results suggest for the first time that upregulated striatal D₃ receptors correlate with TD in non-human primates, adding new insights to the dopamine receptor

*Correspondence should be addressed to: Dr. Pierre Blanchet, Faculty of Dental Medicine, Université de Montréal, P.O. Box 6128, Succ. Centre-ville, Montréal (QC), H3C 3J7, Canada. Phone: (514) 343-7126; pierre.j.blanchet@umontreal.ca.

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Author Roles

S. Mahmoudi: 1) Research project: C; 2) Statistical analysis: B; 3) Manuscript: B.

D. Lévesque: 1) Research project: ABC; 2) Statistical analysis: AB; 3) Manuscript: B.

P. Blanchet: 1) Research project: ABC; 2) Statistical analysis: C; 3) Manuscript: A.

supersensitivity hypothesis. The D₃ receptor could provide a novel target for drug intervention in human TD.

Keywords

dopamine receptors; antipsychotic drugs; basal ganglia; tardive dyskinesia

Introduction

Tardive dyskinesia (TD) is an unsolved and potentially disabling condition encompassing all delayed, persistent, and often irreversible abnormal involuntary movements arising in a fraction of subjects during long-term exposure to centrally-acting dopamine receptor-blocking agents such as antipsychotic drugs (APDs) and metoclopramide.¹ It remains prevalent worldwide even in children,² although a prospective study yielded an adjusted TD incidence rate-ratio one-third less for adults treated with newer-generation (“atypical”) APDs alone compared to first-generation (conventional) agents.³ Treatment options are non specific and produce mixed results.

The pathogenesis of TD has proved complex. Very few, well controlled, clinicopathological studies have been published, and none of the proposed hypotheses, namely dopamine D₂ receptor supersensitivity, striatal neurodegeneration, maladaptive synaptic plasticity, and enhanced serotonin 5-HT₂ receptor signaling, have been satisfactorily confirmed in monkeys and humans.^{4–5} The classic hypothesis of the existence of dopamine D₂ receptor supersensitivity in TD stemmed from rodent studies in which short-term exposure to conventional APDs was almost constantly associated with increased numbers of D₂ receptors^{6–8} and increased mRNA levels.^{8–9} Single administration of haloperidol also enhanced receptor responsiveness to the dopamine agonist apomorphine.¹⁰ Yet those data failed to explain TD occurrence in a fraction of APD-exposed human subjects, and the delay necessary for induction. Protracted (6–8 months) APD treatments in rats have produced increased striatal D₂ binding,¹¹ mRNA levels,¹² and affinity to ligands,¹³ but unchanged D₂ binding has also been reported.¹⁴ Further, in some chronic rat experiments, striatal D₂ receptor density¹⁵ and mRNA levels¹² were found similar between animals with or without oral TD-like movements. Positron emission tomography (PET) studies did not show a difference in D₂ receptor density between APD-treated patients with or without TD,^{16–17} and no post-mortem evidence for striatal dopamine D₂ receptor upregulation was found in TD patients.^{18–20}

In the past decade, the dopamine D₃ receptor has drawn attention as a possible mediator of hyperkinetic movement disorders, including levodopa-induced dyskinesia.^{21–22} Several groups proposed that there might be a genetic predisposition to TD in some ethnic groups carrying the gly9 polymorphism in the dopamine D₃ receptor gene,^{23–24} which reportedly enhances the affinity to dopamine compared to the ser9 receptor.²⁵ This polymorphism is also present in capuchin monkeys used experimentally.²⁶ While traditionally not seen as a prime target of APDs *in vivo* in humans,²⁷ the D₃ receptor displays strong affinity *in vitro* for these drugs, which could then contribute to the response profile. One PET study in baboons using the D₃-preferring ligand (+)-PHNO also suggested that both haloperidol and

clozapine acutely bind to D₃ receptors *in vivo*.²⁸ Behavioural studies conducted in monkeys using partially selective D₃ receptor agents have suggested an involvement of this receptor subtype in TD.²⁹

Given the variability in rodent data, we chronically exposed *Cebus apella* monkeys to typical or atypical APDs to revisit the dopamine receptor supersensitivity hypothesis, in an attempt to correlate TD induction and severity with dopamine D₁, D₂, and D₃ receptor levels measured by autoradiography, a technique allowing detailed anatomic dissection of the basal ganglia and use of a highly D₃-selective ligand unavailable for *in vivo* imaging. We also examined markers of direct and indirect striatal output neurons,^{30–31} as part of an overarching goal to find novel therapeutic targets and drugs that could be readily translated from primates to humans with TD.

Methods and Materials

Animals and treatments

Handling of primates proceeded in accordance to the National Institute of Health Guide for the Care and Use of Laboratory Animals and approval of the local Animal Care Committee. Twenty-three adult ovariectomized female capuchin (*Cebus apella*) monkeys were used, in order to match the endocrine status and purported high TD risk of postmenopausal women. A detailed description of treatments and behavioral measures can be found elsewhere.³² Briefly, 11 animals were injected on a weekly basis with a depot preparation of haloperidol decanoate (0.1–0.9 mg/kg i.m.) (estimated dose between 28–252 mg/month in humans). In the absence of direct pharmacokinetic studies comparing drug handling in capuchins and humans, doses were first selected according to those used by research groups in the field, and individually adjusted to avoid acute dystonic reactions and excessive motor slowing. In the TD group, the mean ($\pm$ SD) latency of onset of TD was 19 ($\pm$ 12) months (range 7–35 months). In the TD-free animals, mean duration of drug exposure was 19 ($\pm$ 4.5) months (range 16–27 months). Six control animals received the atypical APD clozapine at clinically relevant doses, individually titrated to avoid adverse effects (overt sedation or motor slowing), given twice daily at 5–8 mg/kg/dose (estimated dose between 700–1120 mg/day in humans) in a fruit 5 days a week for 6 months (cumulative doses ranging from 600–960 mg/kg). This duration of exposure to clozapine was deemed sufficient to achieve chronic drug impregnation, as this study was not an attempt to compare the dyskinesigenic potential between clozapine and haloperidol. During the last weeks of exposure, the depot haloperidol group was switched to an oral preparation (0.125–0.3 mg/kg twice daily in a fruit) (estimated dose between 16–42 mg/day in humans) in order for all drug-exposed animals to receive their last medication dose 3 h prior to sacrifice. Six animals served as untreated controls.

TD measure

The orofacial (forehead, lips, tongue, jaw), neck, trunk, and limb movements were scored along a primate equivalent of the Abnormal Involuntary Movements Scale, with each body segment scored between 0–4 points from absent to severe TD, for a maximum score of 40 points. A similar scale (ratings between 0–3 points) is used in non-human primate studies of

levodopa-induced dyskinesia.³³ During drug exposure, the presence of TD was deemed unequivocal when persistent over time (>3 months) and a minimum of two separate body segments scored at least 1 point, or a minimum of one body part scored 2 points or more, at least part of the time. Motor rating took place over 15 min each hour for 4 consecutive hours. The cumulative score obtained for all 4 observations is used, and a total score of 0 required for TD-free animals.

Tissue preparation

Following sacrifice and body perfusion with physiologic saline, the brains were removed, immersed in 2-methylbutane at -50°C for 15 sec, and then kept frozen at -80°C . Hemisected brains were cut into coronal sections of 12 μm on a cryostat (-20°C). The slices were thaw-mounted onto SuperFrostPlus (Fisher Scientific Ltd, Nepean, ON, Canada) 75 $\times$ 50 mm slides and stored at -80°C until use.

Receptor autoradiography

[^3H]SCH23390 binding autoradiography to dopamine D_1 receptor was adapted from previous reports with minor modifications.³⁴ Slides were incubated with 2 nM [^3H]SCH23390 (specific activity 91 Ci/mmol; PerkinElmer, Woodbridge, ON, Canada) and non-specific binding was evaluated in the presence of 1 μM SCH23390, as customarily done due to the lack of other D_1 -selective ligands. [^{125}I]iodosulpride binding was used to label dopamine D_2 receptors. [^{125}I]iodosulpride autoradiography protocol was adapted with minor modifications.³⁵ Brain sections were incubated with 0.2 nM [^{125}I]iodosulpride (specific activity 2200 Ci/mmol; PerkinElmer, Woodbridge, ON, Canada). Non-specific binding was evaluated in the presence of 2 μM raclopride. [^{125}I]7-OH-PIPAT binding to dopamine D_3 receptor autoradiography was adapted with minor modifications.³⁶ Brain sections were incubated with 0.2 nM [^{125}I]7-OH-PIPAT (specific activity 2200 Ci/mmol; PerkinElmer, Woodbridge, ON, Canada). Non-specific binding was evaluated in the presence of 1 μM dopamine. Additional information on dopamine receptor autoradiography experimental conditions can be found in supplementary material on the website.

In situ hybridization

Preproenkephalin (PPE) probe preparation—A cDNA oligonucleotide probe complementary to bases 388–435 (GenBank accession no. NM_006211) of the human PPE sequence of the cloned PPE-A cDNA was labeled at the 3'-end by terminal transferase (New England Biolabs, Whitby, ON, Canada) with a [^{35}S]-dATP (1250 Ci/mmol, PerkinElmer, Woodbridge, ON, Canada) using a 3'-terminal deoxynucleotidyl-transferase enzyme kit (Amersham Pharmacia Biotech, Baie d'Urfé, QC, Canada). The reaction was carried out at 37°C for 30 min and labeled oligonucleotide was purified on a QIAquick Nucleotide removal Kit (QIAGEN, Inc., Mississauga, ON, Canada).

PPE mRNA hybridization—*In situ* hybridization of the PPE probe was performed as previously described.³⁷ In brief, sections were fixed for 20 min in 4% paraformaldehyde. After pre-hybridization steps, [^{35}S]-labeled oligonucleotide probe was added in the hybridization buffer to reach a concentration of 1×10^7 cpm/slide. The hybridization buffer was composed of 50% formamide, 10% dextran sulfate, 1X Denhardt's solution, 0.25 mg/ml

yeast tRNA, 0.5 mg/ml denaturated salmon sperm DNA, and 4X SSC and incubated at 37°C for 15 h in a humid chamber. After several washings, the slide-mounted tissue sections were exposed to BioMax MR films (Kodak, New Haven, CT) for 15 days at room temperature. The films were developed and autoradiograms analyzed by densitometry.

Quantification of autoradiograms

Quantification of the autoradiograms was performed using computerized analysis (ImageJ 1.45s software, Wayne Rasband, NIH). Digital brain images were taken using a Grayscale Digital Camera (Model CFW-1612M, Scion Corporation, Maryland, USA). Optical gray densities were transformed into nCi/g of tissue equivalent using standard curves generated with ^{14}C - or ^3H -microscales (ARC 146A- ^{14}C or ARC 123A- ^3H standards, American Radiolabelled Chemicals Inc., St-Louis). The brain areas under examination included anterior and posterior parts of the caudate nucleus (CD) and putamen corresponding to Bregma 2.70 to 0.45 (anterior caudate-putamen) and Bregma -6.30 to -8.10 (posterior caudate-putamen), respectively.³⁸ Anterior and posterior putamen regions have been subdivided into medial (PM) and lateral (PL) portions. Quantifications were also performed in nucleus accumbens (Acc), globus pallidus internal segment (GPi) and external segment (GPe), and substantia nigra *pars compacta* (SNc) and *pars reticulata* (SNr) levels. Average levels of labeling for each area were calculated from three to four adjacent brain sections of the same animal. Background intensities were taken in white matter tracts for each section and were subtracted from every measurement.

Double in situ hybridization procedure

The double *in situ* hybridization procedure was performed as described previously,³⁹ with [^{35}S]UTP-labeled D₃ receptors and non-radioactive dioxigenin (Dig)-labeled preprotachykinin (PPT), which produces substance P.⁴⁰ The substance P neuropeptide is expressed in the same cell population as dynorphin and was chosen as a marker of the direct striatal efferent pathway activity because it displays higher basal expression than dynorphin.⁴¹ For the dopamine D₃ receptor probe, a complementary DNA fragment (from nucleotides 167 to 830) was amplified from striatal monkey total RNA. The fragment was then subcloned into pCRII/TOPO plasmid. The antisense cRNA probe was obtained by plasmid linearization with XhoI and polymerization with the SP₆ RNA polymerase in the presence of [^{35}S]UTP (1250 Ci/mmol, PerkinElmer, Woodbridge, ON, Canada). The PPT probe (200 bp fragment from nucleotides 78 to 277) was prepared and Dig-labeled as previously described.³⁹ The Dig cRNA probe (PPT, 0.75 ng/ μl) was added in the hybridization mix with the D₃ receptor radioactive-labeled probe ($4\text{--}5 \times 10^6$ cpm/ μl). After overnight incubation and washing procedures, slides were processed for Dig revelation. When an adequate staining was obtained, reaction was stopped and slides were dipped in LM-1 photographic emulsion (GE Healthcare Life Sciences, Piscataway, NJ) previously melted at 42°C for 3 h. Two months later, emulsion was developed in D-19 developer (3.5 min) and fixed (5 min) in Rapid Fixer solution (Kodak, New Haven, CT). Slides were mounted using a water-soluble mounting medium. Single- and double-labeled cells were visualized and manually counted with an Axio Imager A.1 photo microscope (Carl Zeiss Canada Ltd, Montreal, QC, Canada) at 400X magnification. Neuron counting was performed in 5–10 different fields within the anterior dorsal putamen. Double-labeled cells were identified by the apposition of more than

10 silver grains with the colored product of the Dig reaction. Neuron counting was estimated on 4 different sections obtained from a total of 4 animals per group investigated.

Statistical analysis

Statistical analysis was performed with Prism version 5.0 software (GraphPad Software Inc., San Diego, CA, USA). All data were expressed as group mean $\pm$ S.E.M. Statistical comparison of mRNA levels in controls, haloperidol- and clozapine-treated groups was performed using a one-way analysis of variance (ANOVA). We first performed a test of homogeneity using Bartlett's test, followed by a logarithmic transformation (when Bartlett's test was significant) and finally applied a one-way ANOVA on transformed data. When the ANOVA revealed significant differences, a Tukey's test was performed as *post hoc* analysis. Comparison between dyskinetic and non dyskinetic haloperidol-treated animals was performed using an unpaired t-test with a two-tailed p value. Statistical significance was set at $p < 0.05$.

Results

Five out of 11 capuchins treated with haloperidol developed lasting spontaneous TD. TD scores ($\pm$ SD) have been reported elsewhere (mean of 16.5 ($\pm$ 8.5) points for the group).³² The resulting abnormal purposeless movements were similar to those found in humans and typically mild and stereotyped in nature, involving the oral and axial/limb musculature, at times admixed with dystonic features. The selected doses of clozapine were well tolerated and caused neither acute dystonic reactions nor TD within 6 months.

Effect of antipsychotic drugs on dopamine receptor binding

[³H]SCH23390 specific binding distribution was homogenous throughout anterior and posterior caudate-putamen subterritories (Fig. S1). Chronic haloperidol and clozapine treatments reduced [³H]SCH23390 specific binding to dopamine D₁ receptors in the anterior medial putamen region, whereas clozapine, but not haloperidol, also reduced binding levels in posterior caudate and posterior lateral putamen (Fig. S1).

[¹²⁵I]iodosulpride specific binding levels were high in nucleus accumbens, and all anterior and posterior caudate-putamen areas (Fig. 1A, B). [¹²⁵I]iodosulpride binding displayed a posterior putamen medial-to-lateral gradient of expression (Fig. 1B). Lower [¹²⁵I]iodosulpride specific binding was found in the GPe, whereas almost undetectable labeling was observed in the GPi in untreated animals (Fig. 1B). Chronic APD exposure was associated with caudate-putamen dopamine D₂ receptor binding site levels largely comparable to unmedicated controls (Fig. 1C), regardless of the motor status of the animals. Dopamine D₂ receptor binding capacities were not altered by APD in the GPe (Fig. 1C), but a strong haloperidol-induced idosulpride binding capacity upregulation was observed in the GPi (CTL = 100 $\pm$ 12%; HAL = 226 $\pm$ 9% [$p < 0.01$ vs CTL group]; CLZ = 97 $\pm$ 39%).

[¹²⁵I]7-OH-PIPAT binding displayed an anterior-to-posterior gradient of labeling in caudate-putamen subterritories in control animals (Fig. 2A, B). Within posterior putamen, [¹²⁵I]7-OH-PIPAT specific binding capacity was found higher medially than laterally (Fig. 2B). This gradient is reverse to that observed with [¹²⁵I]iodosulpride. Thus, receptor distribution

is consistent with the notion that [125 I]iodosulpride binds to both D₂ and D₃ receptors, with a D₂:D₃ ratio more prominent in the anterior striatum of untreated animals in our experimental conditions. High levels of [125 I]7-OH-PIPAT specific binding were observed in the GPi in untreated animals (Fig. 2B). [125 I]7-OH-PIPAT specific binding distribution is also consistent with previous reports on the distribution of dopamine D₃ receptors in both monkeys and humans.⁴² Unlike clozapine, haloperidol strongly upregulated [125 I]7-OH-PIPAT specific binding to dopamine D₃ receptors in all regions examined relative to controls (Fig. 2C). We also compared [125 I]7-OH-PIPAT specific binding in haloperidol-treated animals with or without TD. Monkeys with TD had significantly higher D₃ receptor binding capacities compared to TD-free animals in the anterior striatal areas examined, but not posteriorly (Fig. 2C, insets). Interestingly, D₃ receptor levels also correlated with TD intensity in the lateral putamen area (Fig. 3) but not with total duration of drug exposure, given the goodness of fit obtained (calculated $r^2=0.064$) and lack of a significantly different slope from zero ($p=0.51$) among all haloperidol-exposed monkeys. [125 I]7-OH-PIPAT binding levels were also upregulated in both GP segments, GPi (Fig. 2C) and GPe (CTL = $100 \pm 9\%$; HAL = $219 \pm 31\%$ [$p<0.01$ vs CTL group]; CLZ = $148 \pm 3\%$). In SNr, both haloperidol and clozapine significantly upregulated [125 I]7-OH-PIPAT specific binding (Fig. S2D–F available on website).

Localization of dopamine D₃ receptors in striatal cell subpopulations

We measured localization of the dopamine D₃ receptor mRNA in PPT-negative and PPT-positive cells of the striatum using a double *in situ* hybridization procedure in which the D₃ receptor 35 S-labeled probe can be visualized by silver grain accumulations, whereas the PPT Dig-labeled probe appears as cellular brown deposits (Fig. 4A). Quantification of single Dig-labeled cells (PPT-positive cells) in controls and haloperidol-treated animals indicated similar PPT mRNA levels in both experimental conditions (Fig. 4B). In the anterior lateral putamen area, haloperidol significantly increased the number of cells expressing D₃ receptor mRNA (Fig. 4C), and selectively increased (3.3-fold) the fraction of D₃ receptor labeling in PPT-positive cells (Fig. 4D). Haloperidol treatment did not modulate the D₃ receptor labeling in PPT-negative cells (Fig. 4D).

Effect of antipsychotic drugs on enkephalin expression

[35 S]-PPE-labeled probe showed strong signal throughout nucleus accumbens and caudate-putamen subterritories (Fig. 5A, B). The PPE mRNA signal, expressed in indirect striatopallidal output neurons, was increased by haloperidol and clozapine in all striatal subregions (Fig. 5C). However, haloperidol-exposed animals displayed significantly higher PPE mRNA levels compared to clozapine-treated monkeys in the putamen (Fig. 5C). An additional analysis of the haloperidol group indicated that TD monkeys expressed lower PPE mRNA levels compared to TD-free animals in the posterior dorsolateral putamen (No-Dysk = 263 ± 35 and Dysk = 159 ± 23 nCi/g tissue, $p=0.0352$).

Discussion

Chronic APD impregnation in capuchins caused distinct changes on dopamine D₂-like receptors. In accordance with some rodent data,¹⁴ striatal D₂-labeled [125 I]iodosulpride

binding sites were found at comparable levels between unmedicated and chronically medicated capuchins under haloperidol, regardless of TD status. Evidently, these results should not be construed as reflecting lack of regulation of dopamine D₂ receptors by APDs, but provide no support for the dopamine D₂ receptor supersensitivity hypothesis of TD. However, in stark contrast to previous protocols of APD exposure conducted in rats that yielded no D₃ receptor upregulation in the motor striatum, with mRNA levels^{8–9,43} and binding density^{8,11} generally found unchanged, we observed for the first time in monkeys that long-term haloperidol exposure causes a robust upregulation in D₃ receptors in striatonigral neurons, selectively in the presence of TD. This increase also correlates with TD intensity in the lateral putamen, not with the total duration of haloperidol exposure. Interestingly, chronic clozapine administration spared D₃ receptors, but we acknowledge the fact that the duration of exposure (6 months) may be insufficient to be conclusive in that regard. The foregoing results obtained with haloperidol raise an important caveat in the reliance of experimental TD data restricted to rodents, and the possibility of fundamental inter-species differences in terms of D₃ receptor biology. Methodological issues alone are unlikely involved, given that D₁ receptor binding results following APD exposure appear not so dissimilar between rats and monkeys.

We also found the D₃ receptor widely distributed in striatal and extrastriatal regions, in agreement with previous results obtained in monkeys,⁴⁴ in human post-mortem brain tissues,⁴² and in PET-based *in vivo* brain imaging using the D₃-preferring ligand (+)-PHNO.⁴⁵ Strong basal expression of D₃ receptor binding sites (labeled with [¹²⁵I]7-OH-PIPAT) in the GPi is consistent with localization along the direct striatonigral output pathway, given the preferential expression of D₃ receptor mRNA in PPT-positive cells. Very low levels of iodosulpride binding were observed in the GPi, as opposed to 7-OH-PIPAT binding, in untreated animals. To our surprise, haloperidol induced strong iodosulpride binding in the GPi, perhaps reflecting D₃ rather than D₂ receptor labeling. Such explanation would agree with primate PET imaging data suggesting that the (+)-PHNO signal in the GP mainly represents binding at the dopamine D₃ receptor subtype.^{46–47}

Importantly, haloperidol upregulated D₃ receptors selectively in PPT-positive neurons of origin of the direct striatal efferent pathway. While haloperidol modulates molecular components in cells expressing D₂ receptors,^{48–50} the drug also displays affinity for D₃ receptors, which are mainly co-localized with D₁ receptors in the striatum.⁵¹ It is still unclear how dopamine D₃ receptor upregulation might contribute to TD at the cellular or circuit level, but D₃ receptors have been associated with behavioral sensitization^{52–53} and levodopa-induced dyskinesia,^{21,54} possibly suggesting common mechanisms. Furthermore, overactivation of the dopamine D₁-expressing direct striatonigral pathway has been linked with hyperkinetic movements.^{52,55} Although, studies have generally found no correlation between dopamine D₁ receptors labeling or gene expression and oral TD-like movements in rats,^{11,56–57} our study indicates that clozapine reduced D₁ receptor binding sites in the posterior putamen, a finding of unknown significance at present, but of potential interest in view of the antidyskinetic properties of the drug in humans.^{58–59}

Finally, despite the apparent lack of variations in D₂ receptor density between our experimental groups, APDs still cause distinct changes along the indirect striatal efferent

pathway. For instance, haloperidol, not clozapine, upregulated striatal adenosine A2a receptors in rats.⁶⁰ Both typical (haloperidol) and atypical (clozapine) APDs enhanced enkephalin mRNA levels in all the striatal regions we examined, but to a larger magnitude in the haloperidol group, as reported previously.^{30,61–62} Importantly, this elevation was more noticeable in TD-free animals. In the past, striatal enkephalin overexpression resulting from APDs in rats has been interpreted as reflecting increased activity along the indirect striatopallidal pathway contributing to TD.⁶³ Previous observations generated in murine and primate models of TD suggested that the nuclear receptor *Nur77* is induced in central dopaminergic pathways as part of a neuroadaptive response.³² Indeed, *Nur77* knockout mice displayed enhanced vacuous chewing to haloperidol compared to wild-type littermates,⁶⁴ and TD monkeys had significantly lower *Nur77* mRNA levels than TD-free animals.³² While haloperidol-induced *Nur77* expression is selectively observed in enkephalin-expressing cells of the striatum,³¹ haloperidol-induced enkephalin expression is totally blunted in *Nur77* knockout mice, suggesting that this neuropeptide is a target of *Nur77*-dependent transcriptional activity. Thus, it is tempting to speculate that the rise in enkephalin mRNA levels along the indirect striatopallidal pathway might be part of the adaptive response mounted against TD, as proposed for experimental levodopa-induced dyskinesia.⁶⁵

In conclusion, the foregoing results suggest that the dopamine receptor supersensitivity hypothesis of TD should not entirely be dismissed, but that upregulated striatal D₃ not D₂ receptors are associated with the motor complication in a non-human primate model. The D₃ receptor could provide a novel target for drug intervention in human TD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

[¹²⁵I]iodosulpride binding at D₂ receptors. **A)** The left panels illustrate schematic representations of monkey brain coronal sections taken at anterior (top) and posterior (bottom) levels of the basal ganglia subdivision areas used for quantification. **B)** Representative autoradiograms in anterior (top panels) and posterior (bottom panels) of [¹²⁵I]iodosulpride binding in unmedicated control (CTL), haloperidol (HAL)- and clozapine (CLZ)-exposed groups. **C)** Histograms showing binding results expressed in percentages relative to CTL group values. (**p*<0.05 vs CTL group). Abbreviations: Acc, nucleus accumbens; CD, caudate nucleus; PM, medial putamen; PL, lateral putamen; GPe, external globus pallidus.

Figure 2. $[^{125}\text{I}]7\text{-OH-PIPAT}$ binding at D_3 receptors. **A)** Coronal sections taken as in figure 1. **B)** Representative autoradiograms from controls (CTL), haloperidol-treated (HAL) and clozapine-treated (CLZ) animals. **C)** Histograms showing binding results expressed in percentages relative to CTL group values (** $p < 0.01$ and *** $p < 0.001$ vs CTL groups). Insets: comparison of $[^{125}\text{I}]7\text{-OH-PIPAT}$ binding in haloperidol-treated monkeys without (No-dysk) and with (Dysk) tardive dyskinesia (* $p < 0.05$ vs No-dysk respective group). Abbreviations: Acc, nucleus accumbens; CD, caudate nucleus; PM, medial putamen; PL, lateral putamen; GPi, internal globus pallidus.

Figure 3.

Linear regression curve showing correlation between [¹²⁵I]7-OH-PIPAT binding (nCi/mg tissue) in lateral putamen (PL) and tardive dyskinesia scores in 5 monkeys chronically exposed to haloperidol. Regression analysis indicates that the correlation explains about 80% of the variance in the group.

Figure 4.

Double *in situ* hybridization procedure with [³⁵S]UTP-labeled D₃ receptors and non-radioactive dioxigenin (Dig)-labeled preprotachykinin (PPT), which produces substance P. Neuron counting was measured in 5–10 different fields within the anterior dorsal putamen, on 4 different sections obtained from a total of 3 animals per group. **A)** Examples of D₃-labeled cell (black arrow), PPT-labeled cell (white arrow), and double-labeled D₃/PPT cells (arrowheads) identified by the apposition of more than 10 silver grains with the colored product of the Dig reaction (photograph magnification 400X). **B)** Histograms showing number of single-labeled cells/mm² for the PPT probe in controls (CTL) and haloperidol-treated (HAL) animals. **C)** Histograms showing number of single-labeled cells/mm² for the D₃ receptor probe in controls (CTL) and haloperidol-treated (HAL) animals (**p* < 0.05 vs CTL group). **D)** Histograms showing the percentage of D₃ receptor-labeled cells in PPT⁻-cells and PPT⁺-cells in controls (CTL) and haloperidol-treated (HAL) animals (**p* < 0.05 vs CTL SP⁺-cells group).

Figure 5.

Preproenkephalin (PPE) mRNA hybridization. **A)** Coronal sections taken as in figure 1. **B)** Representative autoradiograms obtained from untreated controls (CTL), haloperidol-treated (HAL) and clozapine-treated (CLZ) animals. **C)** Histograms showing densitometry results expressed in percentages relative to CTL group values (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs CTL respective groups; # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ vs HAL group). Abbreviations: Acc, nucleus accumbens; CD, caudate nucleus; PM, medial putamen; PL, lateral putamen.