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European Journal of Pharmacology 378 (1999) 259–263

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Short communication

Dopamine D_1 receptor mRNA and receptor levels in the striatum of MPTP monkeys chronically treated with SKF-82958

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Received 28 June 1998; accepted 2 July 1998

Abstract

The density of dopamine D_1 receptor antagonist sites was measured by autoradiography and dopamine D_1 receptor mRNA levels were measured by in situ hybridization in the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-exposed monkeys chronically treated with the dopamine D_1 receptor agonist 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide (SKF-82958) administered in intermittent or continuous mode for a month. Normal and MPTP-exposed but otherwise untreated animals were used for comparison. Intermittent treatment with SKF-82958 relieved parkinsonian features and induced dyskinesias whereas given continuously this drug induced behavioral tolerance without dyskinesias. On the one hand, MPTP treatment tended to increase dopamine D_1 receptor density in the putamen whereas treatment of MPTP monkeys with SKF-82958, intermittent or continuous, produced a significant increase compared to control animals. On the other hand, dopamine D_1 receptor mRNA levels in the putamen appeared to decrease after MPTP lesion and agonist treatment as compared to dopamine D_1 receptor density. In contrast, an apparent decrease in dopamine D_1 receptor density and mRNA levels was observed in the nucleus accumbens of untreated MPTP monkeys whereas treatment of MPTP monkeys with SKF-82958, intermittent or continuous, produced a significant decrease compared to control animals. Thus, neither dyskinesias nor tolerance can be exclusively related to an increase or decrease in striatal dopamine D_1 receptors, respectively.

Keywords: Dyskinesia; Autoradiography; Hybridization; Dopamine D_1 receptor; MPTP; Parkinson's disease

1. Introduction

Long-term management with L-3,4-dihydroxyphenylalanine (L-dopa) is plagued by dyskinesias in a majority of Parkinson's disease patients (Nutt, 1990). As alternative for L-dopa treatment, synthetic dopamine receptor agonists were developed. However, given the limitations of treatments with dopamine D_2 receptor agonists which also induce dyskinesias in drug-naive parkinsonian animals (Gomez-Mancilla and Bédard, 1992; Luquin et al., 1992), the therapeutic potential of dopamine D_1 receptor agonists was also investigated. Not only anti-parkinsonian activity provided by dopamine D_1 receptor agonism was observed in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned primates challenged with selective dopamine D_1 receptor agonists (Blanchet et al., 1993; Vermeulen et al., 1993; Grondin et al., 1997), but in animals with L-dopa-induced dyskinesias, dopamine D_1 receptor agonists were less likely to reproduce dyskinesias than with either dopamine D_2 receptor agonists or subsequent challenge of L-dopa (Blanchet et al., 1993; Grondin et al., 1997). In addition to the subtype of dopamine receptors targeted, the mode of stimulation of dopamine receptors is also of importance. Indeed, continuous dopaminergic stimulation is regarded to better regulate basal ganglia circuits and

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0014-2999/99/$ - see front matter
PII: S0014-2999(99)00482-3

improve motor functioning in parkinsonian patients with response fluctuations to standard intermittent oral L-dopa treatment (Sage et al., 1988; Mouradian et al., 1990).

Thus, in attempts to better delineate the optimal way to stimulate dopamine D_1 receptors, we compared continuous and intermittent treatments with the short-acting full dopamine D_1 receptor agonist, 6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide (SKF-82958) in parkinsonian drug-naive monkeys over a month (Goulet et al., 1996). We reported that an intermittent treatment with SKF-82958 relieved in a sustained manner the parkinsonian features in all animals but also induced dyskinesias. However, monkeys receiving continuous treatment with SKF-82958 showed no clear antiparkinsonian response and no dyskinesias despite similar plasma drug levels compared to those treated in an intermittent fashion, suggesting desensitization of dopamine D_1 receptors. In order to associate the behavioral effects of SKF-82958 (dyskinesias or tolerance) with brain biochemical alterations in the striatum, we have measured the density of dopamine D_1 and D_2 receptor antagonist sites by autoradiography and dopamine D_1 and D_2 receptor mRNA levels by in situ hybridization.

2. Materials and methods

2.1. Animals and treatments

Twelve drug-naive cynomolgus monkeys (*Macaca fascicularis*) were used in this study. Three animals received no treatment and served as normal controls. Nine monkeys received weekly doses of MPTP (2 mg per s.c. injection) until a stable parkinsonian syndrome developed, as defined by an unchanged disability score of five or more (maximum of 10) over at least a month according to a disability scale we have used in several published studies (Blanchet et al., 1993; Grondin et al., 1997). Six of these animals received the experimental drug whereas three served as untreated MPTP controls. The dopamine D_1 receptor agonist, SKF-82958 (RBI, Natick, MA, USA), was administered in a continuous ($n = 3$) or intermittent ($n = 3$) mode for 30 days. The intermittent group received thrice-daily s.c. injections of 1 mg kg^{-1} $dose^{-1}$ at 4 h intervals. On day 0, monkeys of the continuous group were anesthetized and Alzet osmotic pumps (Model 2ML1, Alza, Palo Alto, CA, USA) were implanted s.c. in the back of the animals. The pumps delivered SKF-82958 at an infusion rate of 0.125 mg kg^{-1} h^{-1}, roughly equivalent to daily dosage with the intermittent treatment. As previously reported, plasma drug samples were collected from all animals. Plasma drug levels were similar between animals treated intermittently (25–50 ng ml^{-1}) and continuously (23–43 ng ml^{-1}) with SKF-82958 (Goulet et al., 1996).

2.2. Dopamine concentrations, dopamine receptor density and mRNA levels

At the end of the behavioral study, the animals were euthanized after a drug washout period of three days, at which point the parkinsonian features were back to baseline levels corresponding to pretreatment with saline. The brains were quickly removed, hemisected and snap frozen in isopentane (−40°C). The right hemisphere was then cut into coronal sections (12 μm) on a cryostat (−18°C) and striatal dissections were used for dopamine level determination. The slices were thaw-mounted onto polylysine-coated slides and stored at −80°C until processing for biochemical measurements.

Striatal tissues were assayed for dopamine concentrations by high-performance liquid chromatography with electrochemical detection as previously reported (Di Paolo et al., 1986). Density of dopamine D_1 and D_2 receptor antagonist sites was measured by autoradiography using [^{3}H]SCH-23390 (Amersham; 83 Ci $mmol^{-1}$) for dopamine D_1 receptor and [^{3}H]spiperone (Amersham; 89 Ci $mmol^{-1}$) for dopamine D_2 receptor binding in the caudate–putamen and nucleus accumbens as previously described (Gagnon et al., 1990). Ketanserine (50 nM) was added to block binding to serotonin (5-HT_2) receptors. In situ hybridization histochemistry was performed in the same areas with human cDNA corresponding to base pairs 483–1216 for dopamine D_1 receptors and to base pairs 651–1056 for dopamine D_2 receptors as previously described (Goulet et al., 1997).

2.3. Data analysis

Levels of autoradiographic labeling in the nucleus accumbens and in the medial and lateral halves of the caudate–putamen were quantified by computerized densitometry (NIH Image 1.6) at rostral (A17–A20) and caudal (A12–A15.5) levels (Szabo and Cowan, 1984). For autoradiography, total and non-specific binding were measured in adjacent sections and specific binding was obtained by subtracting non-specific from total binding. Standard curves were generated from tritium strips and used to convert optical densities in fmol mg^{-1} of tissue. For in situ hybridization, a standard grayscale strip was used to generate a calibration curve for optical densities. The background was subtracted for each sections with a measure from a region without labeling. The investigator was blind to group membership during analysis as the slides were coded. The measured values were analyzed with a One-way Analysis of Variance followed by a post-hoc analysis with the Fisher probability of least significant difference (PLSD) test. The data were later expressed as relative percentage difference from the mean of the control animals for illustration purposes. Comparison of labeling between normal, untreated MPTP and SKF-82958-treated animals was carried out from sections processed at the same time.

3. Results

Striatal dopamine levels were markedly and similarly reduced between SKF-82958-treated (intermittent and continuous) and untreated MPTP animals. For instance dopamine concentrations of untreated MPTP and SKF-82958-treated animals were 4.1 ± 1.8% (MPTP), 3.4 ± 1.2% (intermittent) and 9.7 ± 6.7% (continuous) of control value (111 ± 15 ng mg^{-1} of protein) in the putamen and 3.2 ± 1.2% (MPTP), 4.8 ± 1.0% (intermittent) and 2.8 ± 1.7% (continuous) of control value (125 ± 14 ng mg^{-1} of protein) in the caudate nucleus ($P < 0.01$ vs. control, not shown).

MPTP treatment tended to increase dopamine D_1 receptor density in the caudate nucleus (up to +13% on average, in the lateral part at rostral level) with virtually no significant changes following chronic treatments with SKF-82958 (not shown). Similarly, as shown in Fig. 1, MPTP treatment tended to increase dopamine D_1 receptor density (up to +15% on average) in the lateral (Fig. 1A) and medial (Fig. 1B) putamen at both rostral and caudal levels. However, MPTP-exposed animals chronically treated with SKF-82958, either intermittent (dyskinesias) or continuous (tolerance), showed a significant increase in dopamine D_1 receptor density compared to control animals, particularly in the lateral putamen (from +20% to 30% on average). On the other hand, dopamine D_1 receptor mRNA levels in the putamen tended to change (decrease) in opposite direction after MPTP lesion and SKF-82958 treatments as compared to dopamine D_1 receptor binding density (Fig. 1C–D). Similar changes in dopamine D_1 receptor expression were observed in the caudate nucleus (not shown).

In contrast to what we observed in the putamen, an apparent decrease in dopamine D_1 receptor density (−10% on average) and mRNA levels (−25% on average) was seen in the nucleus accumbens of untreated MPTP-monkeys vs. control animals (Fig. 2). MPTP-exposed animals chronically treated with SKF-82958, either intermittent (dyskinesias) or continuous (tolerance), showed a significant decrease in dopamine D_1 receptor density (−30% on average) compared to control animals (Fig. 2). A significant decrease of dopamine D_1 receptor mRNA levels was also observed in the nucleus accumbens of SKF-82958-treated animals (−45% on average), regardless of the mode of administration (Fig. 2). No changes in either dopamine D_2 receptor binding density or dopamine D_2 receptor mRNA levels were observed in SKF-82958-treated

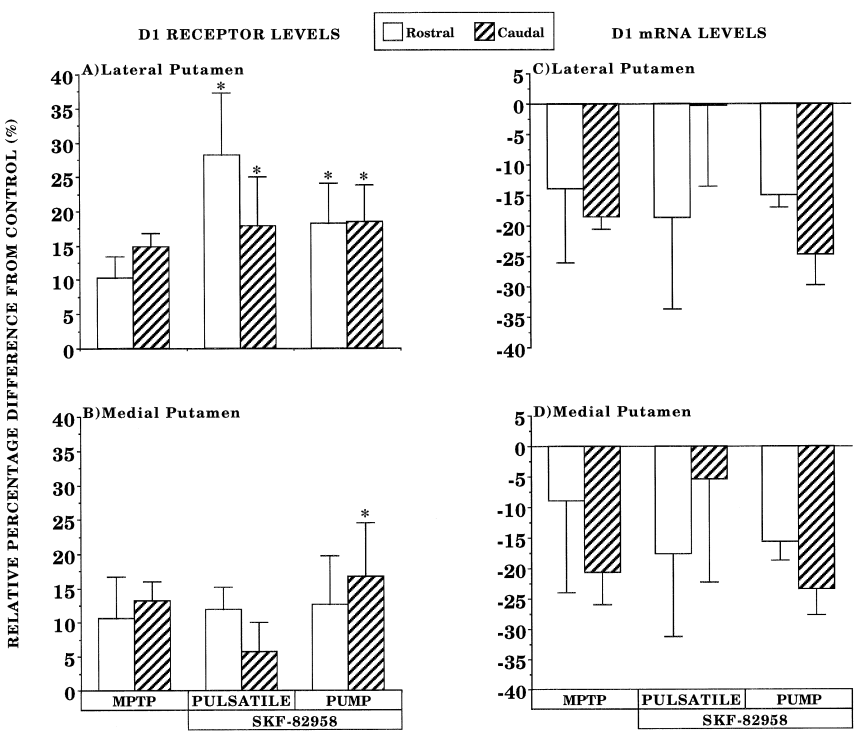

Fig. 1. Effects of MPTP lesioning and chronic treatments of MPTP-exposed monkeys with the dopamine D_1 receptor agonist SKF-82958 on putamen dopamine D_1 receptor density and mRNA levels as measured at rostral and caudal levels by autoradiography of [^{3}H]SCH 23390 binding and in situ hybridization, respectively. Putamen control values for dopamine D1 receptor levels (fmol mg^{-1} of tissue) and for D1 mRNA levels (optical density) were respectively: lateral putamen rostral, 592.3 ± 41.5 and 0.125 ± 0.021; lateral putamen caudal, 513.7 ± 1.2 and 0.101 ± 0.014; medial putamen rostral, 518.1 ± 32.7 and 0.118 ± 0.014; medial putamen caudal, 424.6 ± 10.7 and 0.087 ± 0.010. Values shown are expressed as relative percentage difference ± S.E.M. from the mean of control animals. *$P < 0.05$ versus control (ANOVA + Fisher PLSD).

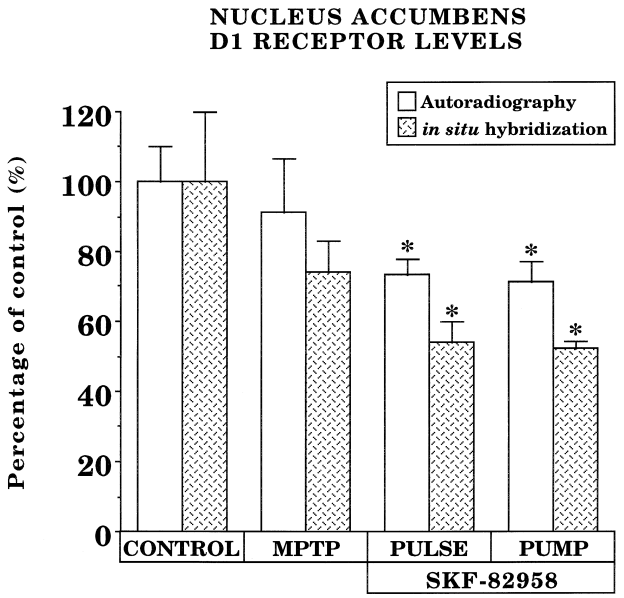

Fig. 2. Effects of MPTP lesioning and chronic treatments of MPTP-exposed monkeys with the dopamine D_1 receptor agonist SKF-82958 on nucleus accumbens dopamine D_1 receptor density and mRNA levels as measured by autoradiography of [^{3}H]SCH 23390 binding and in situ hybridization, respectively. Nucleus accumbens control values were: 296.0 ± 6.4 fmol mg^{-1} of tissue and 0.127 ± 0.031 (optical density) for autoradiography and for mRNA levels, respectively. Values shown are expressed as relative percentage difference $\pm$ S.E.M. from the mean of control animals. $^*P < 0.05$ versus control (ANOVA + Fisher PLSD).

animals compared to control or untreated MPTP monkeys (not shown).

4. Discussion

The present results indicate that SKF-82958 is selective for dopamine D_1 receptors in vivo in that it failed to alter dopamine D_2 receptor binding and mRNA levels at the dosage used. This is consistent with in vitro binding studies on SKF-82958 (Andersen and Jansen, 1990) which have revealed a 176-fold selectivity for dopamine D_1 vs. D_2 receptors (K_i of 0.5 and 88 nM, respectively). In addition, all MPTP-exposed animals were extensively and similarly denervated, suggesting that dopamine D_1 receptor density and mRNA changes observed following SKF-82958 administration were treatment related.

The opposite changes in dopamine D_1 receptor density in the putamen and nucleus accumbens following dopamine D_1 receptor agonist treatment suggest that different regulatory mechanisms of dopamine receptors take place between nigrostriatal and mesolimbic neurons. Internalization of dopamine D_1 receptors after activation by dopamine receptor agonists has been recently reported in vivo (Dumartin et al., 1998), which could explain the decrease in dopamine D_1 receptors observed in the nucleus accumbens. Moreover, dopamine D_1 receptor density and mRNA levels appear to be regulated in opposite direction in the dopamine denervated putamen. A similar opposite regulation was previously observed in 6-hydroxydopamine-lesioned rats (Gerfen et al., 1990) and in MPTP-lesioned monkeys (Goulet et al., 1997). It is possible that dopamine D_1 receptor gene transcription returns to a normal state by three days post-treatment such as for the parkinsonian features but that dopamine D_1 receptor protein increase lasts longer. Alternatively, the dopamine D_1 receptor binding changes may be the result of other cellular mechanisms such as a decrease in the rate of receptor degradation and/or internalization. Although not consistent with the increase in dopamine D_1 receptor density measured in the putamen, the lesion-induced decrease of dopamine D_1 receptor mRNA is in accordance with a decrease in density of the agonist site labeled with [^{3}H]SKF-38393 reported for an other group of MPTP monkeys (Gagnon et al., 1995). Similar findings were also observed in the striatum of 6-hydroxydopamine-treated rats where the high-affinity agonist state of the dopamine D_1 receptors as labeled with [^{3}H]dopamine was decreased (Hervé et al., 1992).

In both groups of MPTP-exposed monkeys treated with SKF-82958, dopamine D_1 receptor density in the putamen were increased, but only animals of the intermittent group developed dyskinesias. Thus, dyskinesias could not be specifically associated with an increase of dopamine D_1 receptor density in the putamen. The lack of response of the monkeys receiving a continuous infusion of SKF-82958 is not due to the absence of the drug, since drug levels were comparable for the two groups (Goulet et al., 1996), suggesting desensitization of the dopamine D_1 receptor subtype. However, behavioral tolerance to the continuous administration of SKF-82958 observed in the present study cannot be attributed exclusively to a decrease in striatal dopamine D_1 receptor density or expression, although a decrease of the dopamine D_1 receptor agonist site (Gagnon et al., 1995) and/or its coupling to second messengers is not excluded. In conclusion, neither dyskinesias nor tolerance can be exclusively related to an increase or decrease in striatal dopamine D_1 receptors, respectively.

Acknowledgements

This work was supported by grants from the Parkinson Foundation of Canada (T.D.P. and P.J.B.) and the Medical Research Council of Canada (T.D.P. and P.J.B.). Richard Grondin and Martin Goulet held studentships from Le Fond de la Recherche en Santé du Québec (FRSQ).

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