Anti-dyskinetic effect of anpirtoline in animal models of L-DOPA-induced dyskinesia

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The serotonin system has emerged as a potential target for anti-dyskinetic therapy in Parkinson’s disease. In fact, serotonin neurons can convert L-DOPA into dopamine, and mediate its synaptic release. However, they lack a feedback control mechanism able to regulate synaptic dopamine levels, which leads to un-physiological stimulation of post-synaptic striatal dopamine receptors. Accordingly, drugs able to dampen the activity of serotonin neurons can suppress L-DOPA-induced dyskinesia in animal models of Parkinson's disease.

Here, we investigated the ability of the 5-HT1A/1B receptor agonist anpirtoline to counteract L-DOPA-induced dyskinesia in L-DOPA-primed 6-OHDA-lesioned rats and MPTP-treated macaques. Results suggest that anpirtoline dose-dependently reduced dyskinesia both in rats and monkeys; however, the effect in MPTP-treated macaques was accompanied by a worsening of the Parkinson’s disease score at significantly effective doses (1.5 and 2.0 mg/kg). At a lower dose (0.75 mg/kg), anpirtoline markedly reduced dyskinesia in 4 out of 5 subjects, but statistical significance was prevented by the presence of a non-responsive subject.

These results provide further evidence that the serotonin neurons contribute both to the pro-dyskinetic effect of L-DOPA and to its therapeutic efficacy in the rat and monkey models of Parkinson’s disease.

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1. Introduction

In the recent years, the serotonin system has emerged as a key player in the appearance of L-DOPA-induced dyskinesia (LID) in animal models of Parkinson’s disease (PD) (Carta et al., 2007; Muñoz et al., 2008; Navailles et al., 2010). It is known since early studies that exogenously administered L-DOPA can be taken up by serotonin neurons and converted to dopamine (DA) by the activity of the aromatic amino acid decarboxylase enzyme (Arai et al., 1994, 1995); however, the impact of the serotonin neuron-derived DA release on the therapeutic effect of L-DOPA in PD has only been a subject of recent research (Tanaka et al., 1999; Carta et al., 2007, 2008; Dupre et al., 2008). Interestingly, removal of the forebrain serotonin innervation by the selective toxin 5,7-dihydroxy-tryptamine has been shown to produce an almost complete suppression of LID in the rat 6-hydroxy-dopamine (6-OHDA)-lesioned model of PD (Carta et al., 2007; Eskow et al., 2009). Similarly, pharmacological silencing of serotonin neuron activity by serotonin 5-HT1 receptor agonists was shown to produce a dose dependent suppression of LID in dyskinetic rats (Carta et al., 2007; Muñoz et al., 2009).

Importantly, co-administration of the 5-HT1A and 5-HT1B receptor agonists 8-OH-DPAT and CP-94253, respectively, was found to induce a potent synergistic effect of suppression of LID, with a near-to-complete abolishment at doses that were marginally effective when given individually (Carta et al., 2007). These results have been later reproduced in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkey model...
of PD, where a significant suppression of LID could be seen without compromising the therapeutic effect of L-DOPA (Muñoz et al., 2008).

In light of these results, the serotonin system became an intriguing therapeutic target for the treatment of LID in dyskinetic patients (Carta et al., 2010; Carta and Bézard, 2011). However, clinical application of these promising pre-clinical observations requires the identification of compounds with proper pharmacological and toxicological profile, able to selectively activate both the 5-HT1A and 5-HT1B autoreceptors, so to trigger the aforementioned synergistic effect on dampening serotonin neuron release.

Anpirtoline is a 5-HT1 receptor agonist with higher affinity for the 5-HT1B receptor over the 5-HT1A (Schlicker et al., 1992). Previous experimental studies investigated the antiocepic/antidepressant like action of anpirtoline in rodents (Schlicker et al., 1992). Most interestingly, anpirtoline was also investigated for its analgesic properties in a small group of patients (Hummel et al., 1994). Albeit the drug was not pursued further for this indication, no serious side effects were reported in these patients. Thus, in the present study, we have investigated the anti-dyskinetic effect of anpirtoline in the rat and monkey models of LID, and its impact on the therapeutic efficacy of L-DOPA.

2. Materials and methods

2.1. Rat study

Adult female Sprague–Dawley rats (225–250 g; B&K Universal, Sweden) were housed under a 12 h light/12 h dark cycle with free access to water and food. All surgical procedures were performed according to the regulations set by the Ethical Committee for use of Laboratory animals at Lund University.

2.1.1. Drugs

All the drugs were diluted in 0.9% sterile saline. 6-OHDA and benserazide were purchased from Sigma–Aldrich AB, Sweden. L-3,4-dihydroxyphenylalanine methyl ester hydrochloride (L-DOPA) and amphetamine sulphate were purchased from Research Organics, Cleveland, OH. 6-Chloro-2-[piperidinyl-4-thio]pyridine (6-Chloro-2-[piperidinyl-4-thio]pyridine) and anpirtoline (anpirtoline) was purchased from Tocris Bioscience, UK.

2.1.2. Experimental design

Fifty rats were subjected to 6-OHDA injection into the MFB (14 μg free base in 4 μl in 0.02% L-ascorbic acid in 0.9% saline) in order to achieve a complete lesion of the nigrostriatal pathway, at the following coordinates: AP: −4.4 mm, ML: −1.2 mm, DV: −7.8 mm relative to bregma, according to Paxinos and Watson (1998). The toothbar was set at −2.4 mm. Injection speed was 1.0 μl/min and the syringe was kept in place for an additional 3 min before it was slowly retracted (Carta et al., 2007).

Three weeks later, the rats were screened in the amphetamine-induced rotation test (2.5 mg/kg, i.p.). Animals exhibiting ≥6 full body turns/min ipsilateral to the lesion side were treated with L-DOPA (6 mg/kg plus 10 mg/kg benserazide, s.c.) daily for 3 weeks, until a stable level of abnormal involuntary movements (AIMs) was achieved. The animals were then allocated into four groups, balanced according to their AIMs scores, and then challenged with L-DOPA alone or in combination with anpirtoline at three doses (0.1, 0.5, 2 mg/kg, s.c.).

2.1.3. Behavioral analysis

AIMs were evaluated according to the rat dyskinesia scale as previously described (Lundblad et al., 2002; Carta et al., 2007; Muñoz et al., 2008). Briefly, AIMs were classified into four subtypes according to their topographic distribution as Forelimb (Li), Orolingual (Ol) Axial (Ax), and locomotive (Lo) dyskinesia (displayed as contralateral rotation). The severity of each AIM subtype was assessed using scores from 0 to 4 (1: occasional, i.e. present less than 50% of the time; 2: frequent, i.e. present more than 50% of the time; 3: continuous, but interrupted by strong sensory stimuli; 4: continuous, not interrupted by strong sensory stimuli).

2.1.4. Activity test

Locomotor activity was assessed in a separate group of animals in open-field chambers, each equipped with a 16 × 16 infrared photobeam system using the Flex-Field Software system (San Diego Instruments, San Diego, CA). Animals were habituated for 1 h before the drugs were injected and the measurements begun, as described in Muñoz et al. (2009).

2.1.5. Immunohistochemistry

The animals employed in the chronic study were sacrificed 48 h after the last injection, the brains removed and processed for the tyrosine hydroxylase (TH) immunohistochemistry (to verify the dopaminergic lesion). Rats with less than 90% of dopaminergic depletion (data not shown) were excluded from the study (Carlsson et al., 2007; Carta et al., 2007; Tronci et al., 2012; Ulusoy et al., 2010).

2.2. Monkey study

Five female cynomolgus monkeys (Macaca fascicularis, Xierxin, Beijing, PR of China) were used. Animals were 5 years old on average and their body weights were 3.3 ± 0.3 kg (mean ± SD). They were housed in individual primate cages under controlled conditions of humidity, temperature, and light (12-h light/12-h dark cycle, lights on at 8:00 am); food and water were available ad libitum in an AAALAC-accredited facility. Animal care was supervised by veterinarians skilled in the healthcare and maintenance of non-human primates. Experiments were carried out in accordance with European Communities Council Directive of 24 November 1986 (86/609/EEC) for care of laboratory animals.

2.2.1. Experimental parkinsonism and dyskinesia

Animals had been rendered parkinsonian by daily injection of MPTP hydrochloride (0.2 mg/kg, i.v.) until parkinsonian signs appeared. Following stabilization of the parkinsonian syndrome, L-DOPA therapy commenced and L-DOPA-induced dyskinesia was characterized in detail for each animal. These procedures and methods have been previously described (Bézard et al., 2003, 2013; Guigoni et al., 2005; Muñoz et al., 2008; Porras et al., 2012). Since animals were previously exposed to other treatments for PD or dyskinesia, a minimum of 2-week washout period (with daily exposure to L-DOPA) was given prior to the start of the study, to avoid any possible drug-drug interactions. L-DOPA/carbipoda was administered orally, while anpirtoline was given s.c. After the compound administration, the animals were immediately transferred to an observation cage for a 240-min behavioral assessment. The following treatments were employed: L-DOPA/carbipoda + vehicle, L-DOPA/carbipoda + anpirtoline (0.15, 0.45, 0.75, 1.5 and 2.0 mg/kg).

2.2.2. Behavioral assessment

A battery of behavioral tests was performed as previously described (Bézard et al., 2003, 2013; Guigoni et al., 2005; Muñoz et al., 2008; Porras et al., 2012). A quantitative assessment of locomotor activity using computer-based passive infrared activity monitors (Excalibur, modified by the Central Electronic Workshop, University of Manchester) was obtained every 5 min for 240 min. Non-parametric measures based on range of movement, bradykinesia and posture scales were made, parkinsonian condition (and its reversal) was assessed on a parkinsonian monkey rating scale
by post hoc analysis of video-recordings by an observer blinded to the treatment, in 10-min observation periods every 30 min for 240 min, as previously described (Bézard et al., 2003; Aubert et al., 2005). The severity of dyskinesia was rated using the Dyskinesia Disability Scale (Fox et al., 2012; Bézard et al., 2013) by post hoc analysis of video-recordings, in 10-min observation periods every 30 min for 240 min. Both choreic (hyperkinetic, purposeless dance-like movements) and dystonic (sustained, abnormal muscle contractions) components of dyskinesia were rated as reported previously (Bézard et al., 2003; Aubert et al., 2005). For ethical reasons the monkey were not sacrificed after the pharmacological experiments. However, the same MPTP treatment has been used in several previous studies (Bézard et al., 2013; Guigoni et al., 2005; Muñoz et al., 2008), and was shown to produce near-complete loss of TH-positive cells (Bézard et al., 1997).

2.3. Statistical analysis

Statistical analysis of rat data was performed using Statistica Software. Significance between groups was evaluated by one-way ANOVA followed by Newman–Keuls multiple comparisons test. Behavioral data in the macaque model were analyzed using Friedman followed by Dunn’s multiple comparisons test. Statistical significance was set at p < 0.05.

3. Results

3.1. Effect of anirptoline on L-DOPA-induced dyskinesia and motor activity in 6-OHDA-lesioned rats

6-OHDA-lesioned rats were treated with L-DOPA (6 mg/kg plus 10 mg/kg benserazide, s.c.) for 4 weeks. Dyskinetic rats were allocated to 4 different groups (n = 7/group), balanced according to their AIMS score, to receive L-DOPA only, or in combination with anirpitoline at 3 different doses (0.5, 1.0 and 2.0 mg/kg, s.c.). Results showed that anirptoline produced a dose-dependent reduction of already established LIDs, which was near-to-complete at the highest tested dose (Fig. 1A).

In order to investigate the impact of the anirptoline treatment on the therapeutic effect of L-DOPA, dyskinetic animals were allocated into 4 groups (n = 7/group), to receive treatment with saline, L-DOPA, L-DOPA plus anirpitoline 2.0 mg/kg, or anirpitoline 2.0 mg/kg, and locomotor activity was measured by an automated system. Data showed that the dose of anirptoline of 2.0 mg/kg, which was able to reduce AIMS by about 90%, did not significantly affect L-DOPA-induced motor activation (Fig. 1B).

3.2. Effect of anirptoline on L-DOPA-induced dyskinesia in MPTP-treated dyskinetic macaques

Five dyskinetic MPTP-treated macaques were employed to test the effect of escalating doses of anirptoline on LID and PD score. Results showed that anirptoline produced a dose-dependent anti-dyskinetic effect with full suppression at the highest tested dose (χ²(6) = 15.89, p < 0.001; Fig. 2A and B). However, the anti-dyskinetic effect of the highest doses (1.5 and 2.0 mg/kg) took place at the expense of the therapeutic effect of L-DOPA (Fig. 2C and D). The decrease in dyskinesia is mirrored by an increase in the disability scores (χ²(6) = 19.31, p < 0.001; Fig. 2B and D). Anirptoline reduced in dose-dependent manner the on-time, i.e. time during which the bradykinesia score was equal to 0 (χ²(6) = 20.37, p < 0.001). However, only anirptoline at the dose of 1.5 and 2.0 mg/kg reached significance (p < 0.05). The average on-time for vehicle and anirptoline at the doses of 0.15, 0.45, 0.75, 1.5 and 2.0 mg/kg was 184, 184, 132, 88, 20 and 20 min, respectively. Anirptoline at the highest doses, accordingly, diminished the locomotor activity (χ²(6) = 19.317, p < 0.001; Fig. 2E and F).

4. Discussion

In the present study, the 5-HT1 receptor agonist anirptoline was found to produce a dose-dependent reduction of LIDs, both in the rat and monkey models of PD; however, in the MPTP-treated macaques, this effect was statistically significant only at the two higher tested doses, which also induced a significant worsening of the therapeutic efficacy of L-DOPA. A detailed analysis of the data revealed a high variability in the response of the monkeys to the anirptoline treatment; in one of the five macaques, anirptoline produced a striking anti-dyskinetic effect already at the lower tested dose (0.15 mg/kg), while the number of highly responsive monkeys increased to 2 and 4 for anirptoline 0.45 and 0.75 mg/kg, respectively. The presence of a non-responsive subject prevented the anti-dyskinetic effect of anirptoline 0.75 mg/kg to reach statistical significance; nevertheless, more than 60% reduction of LIDs was seen in four out of five subjects without inducing marked reduction of the L-DOPA effect in reversing the disability score and promoting motor activity. The non-responsive subject was unaffected also by the two higher anirptoline doses.

These results are in line with the increasing body of evidence pointing to the serotonin system as a key player in the induction of LIDs in animal models, as well as with the concept that DA released from serotonin neurons may represent the main source of L-DOPA-derived DA when most of the striatal dopaminergic terminals have degenerated.

Similar results were recently obtained with eltoprazine, another 5-HT1 receptor agonist, in the same animal models (Bézard et al., 2013). Indeed, eltoprazine was also found to completely suppress LID, but at the expense of the PD score in MPTP-treated macaques. Nevertheless, eltoprazine is under clinical investigation in dyskinetic PD patients (see press release at http://www.psychogenics.com/pdf/Positive_Efficacy_Data_in_Levo_dopa_Induced_Dyskinesia.pdf). Compared to eltoprazine, anirptoline has higher affinity for the 5-HT1B receptor over the 5-HT1A (Schlicker et al., 1992). Therefore, this investigation was undertaken to understand whether preferential activation of 5-HT1B receptor subtype could yield more favorable results. In fact, 5-HT1A and 5-HT1B receptors have different topographical distribution as pre-synaptic receptors, with the 5-HT1A preferentially expressed at cell body level, and the 5-HT1B receptor expressed on serotonergic terminals in target areas. Although moderate doses of anirptoline appeared to produce more favorable results compared to eltoprazine (at least in a subset of animals), statistically significant doses worsened the therapeutic effect of L-DOPA in both cases.

It is worth pointing out that 5-HT1A and 5-HT1B receptors are also located post-synaptically in cortical and striatal neurons, where they serve to control glutamate and GABA release, respectively. Activation of these receptors has been suggested to contribute to the anti-dyskinetic and anti-parkinsonian effect of 5-HT1 receptor agonists (Dupre et al., 2008; Bishop et al., 2009; Zhang et al., 2008).

We have previously shown that combination of subthreshold doses of experimental 5-HT1A and 5-HT1B receptor agonists (8-OH-DPAT and CP-94253, respectively) produced significant suppression of dyskinesia without worsening the therapeutic effect of L-DOPA (Muñoz et al., 2008); however, also in that study, administration of higher doses of 5-HT1A and 5-HT1B receptor agonists reduced the therapeutic effect of L-DOPA. These results confirm that in a context of complete DA denervation, the therapeutic effect of L-DOPA appears to be dependent on DA released from serotonin terminals; therefore, excessive inhibition of serotonin.
Fig. 1. (A) Effect of anpirtoline (anp) on L-DOPA-induced dyskinesia in the 6-OHDA rat model of LID. One-way ANOVA followed by Newman–Keuls multiple comparison test revealed that anpirtoline produced a dose-dependent reduction of already established LIDs, which was near-to-complete at the higher tested dose (**p < 0.001 vs LD, \\(* * * p < 0.001 vs LD + anp 0.5, * * p < 0.05 vs LD 6 + anp 1.0; n = 7/group). (B) Effect of anpirtoline on L-DOPA-induced motor activation in the 6-OHDA rat model of LID. One-way ANOVA followed by Newman–Keuls multiple comparison test revealed that anpirtoline 2.0 mg/kg did not reduce the ability of L-DOPA to improve motor activity (**p < 0.001 vs saline, * * * p < 0.01 vs saline, * p < 0.05 vs LD 6; n = 7/group).

Fig. 2. Effect of anpirtoline on dyskinesia, disability and locomotor activity in the MPTP-lesioned macaque model of LID. For each parameter, the time-course and the cumulated data over the 4 h of observation are presented. Data are shown as median for dyskinesia (A and B) and disability (C and D). Data are presented as mean for the locomotor activity count (E and F). Cumulated data were analyzed using Friedman’s test followed by Dunn’s multiple comparison. SEM values have been omitted in all graphs for clarity (*p < 0.05, **p < 0.01, vs vehicle; n = 5/group).
neurons should be avoided not to block the beneficial effects. Thus, clinical efficacy of this approach for treatment of LIDs may be dependent on the identification of a compound, with proper affinity ratio for the 5-HT1A and 5-HT1B receptors, able to produce a moderate damping of serotonin neuron release.

The narrow therapeutic window represents a concern for clinical application of this approach, and may require a careful titration of the candidate drug in each individual patient. It is also possible that 5-HT1 receptor agonists might provide a more efficacious application in a subset of patients retaining some residual striatal dopaminergic innervation, which is expected to contribute to the therapeutic effect of L-DOPA by providing a source of regulated DA release, and also buffer serotonin neuron-derived DA. Therefore, 5-HT1 receptor agonists may be more effective against moderate dyskinesias seen in earlier stages of disease, and may also prevent the appearance of troublesome dyskinesia. Clinical studies are warranted to verify this hypothesis, as well as to investigate the impact of 5-HT1 receptor activation on mood, given that this is also expected to transiently reduce serotonin release.

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