Experimental Neurology 219 (2009) 298–307

Contents lists available at ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Serotonin neuron-dependent and -independent reduction of dyskinesia by 5-HT_{1A} and 5-HT_{1B} receptor agonists in the rat Parkinson model

Ana Muñoz [a,c], Thomas Carlsson [a,b,1], Elisabetta Tronci [a], Deniz Kirik [b], Anders Björklund [a], Manolo Carta [a,b,*]

[a] Neurobiology Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, Lund, Sweden
[b] Brain Repair and Imaging in Neural Systems (B.R.A.I.N.S.) Unit, Department of Experimental Medical Science, Lund University, Lund, Sweden
[c] Department of Morphological Sciences, University of Santiago de Compostela, and CIBERNED, Spain

ARTICLE INFO

Article history:
Received 12 March 2009
Revised 20 May 2009
Accepted 22 May 2009
Available online 3 June 2009

Keywords:
Parkinson's disease
L-DOPA
Dyskinesia
AIMs
Serotonin
5-HT_{1A} agonist
5-HT_{1B} agonist
Apomorphine
Glutamate
GABA

ABSTRACT

5-HT_1 receptor agonists have been shown to reduce abnormal involuntary movements (AIMs) in the rat and monkey models of L-DOPA-induced dyskinesia. Different mechanisms have been proposed to underlie this effect. Activation of pre-synaptic 5-HT_1 receptors has been suggested to inhibit dysregulated release of dopamine from the serotonin terminals, and thus, abnormal activation of striatal dopamine receptors. Activation of post-synaptic 5-HT_1 receptors expressed in non-serotonergic neurons in different brain areas, by contrast, has been shown to result in decreased glutamate and GABA release, which may also contribute to the antidyskinetic effect.

To unveil the relative contribution of these mechanisms, we have investigated the effect of increasing doses of 5-HT_{1A} and 5-HT_{1B} receptor agonists on AIMs induced by either L-DOPA or apomorphine. In contrast to L-DOPA-induced AIMs, which were dampened already at low doses of 5-HT_1 agonists, reduction of apomorphine-induced AIMs required higher doses. Removal of the serotonin innervation suppressed L-DOPA-induced AIMs, but neither affected apomorphine-induced AIMs nor the inhibiting effect of 5-HT_1 agonists on AIMs induced by the direct dopamine agonist, suggesting that such effect is independent on activation of pre-synaptic 5-HT_1 receptors.

Introduction

The serotonin system is emerging as a key element in the development of dyskinesia induced by long-term L-DOPA treatment in parkinsonian rats and monkeys. The serotonin neurons are known to express the aromatic amino acid decarboxylase (AADC) and the vesicular monoamine transporter (VMAT), which mediate conversion of L-DOPA to dopamine (DA) and storage into synaptic vesicles, respectively. Indeed, Tanaka et al. have shown that removal of the serotonin innervation led to about 80% reduction in L-DOPA-derived DA in the striatum of 6-hydroxydopamine (6-OHDA)-lesioned rats (Tanaka et al., 1999). Moreover, L-DOPA-derived DA release appears to rely on vesicular storage, since pretreatment of 6-OHDA-lesioned rats with reserpine has been shown to reduce L-DOPA-derived extracellular DA levels by more than 80% (Kannari et al., 2000).

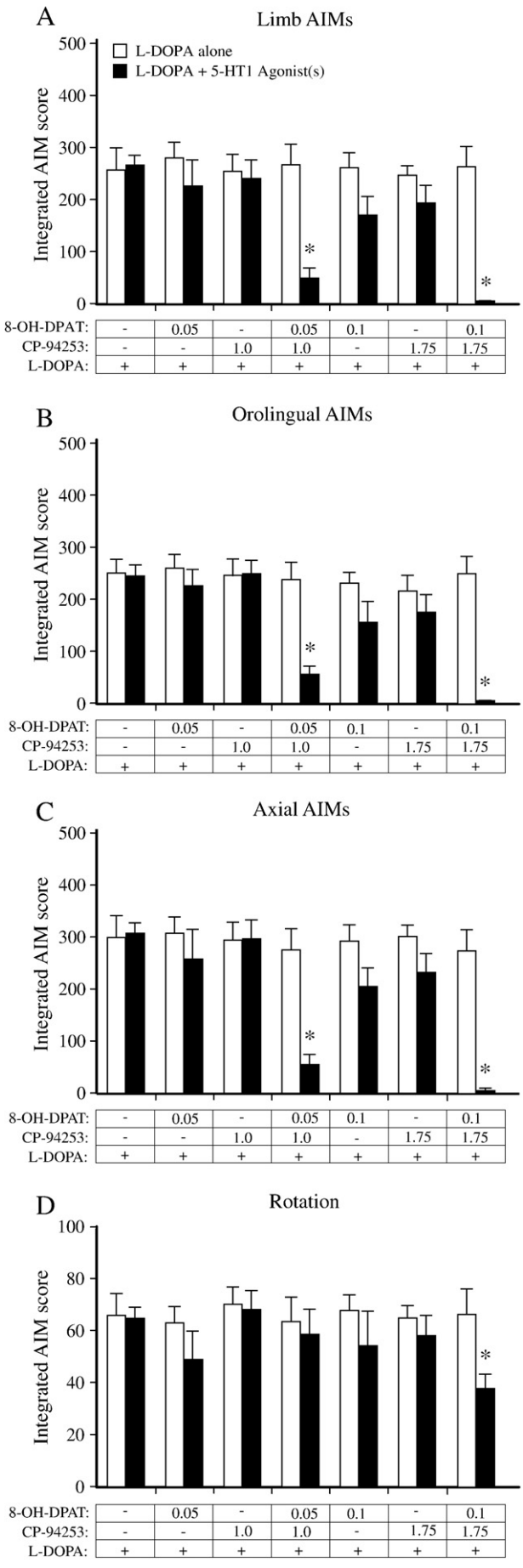
We have recently shown that serotonin neuron-dependent DA release has a pro-dyskinetic effect (Carta et al., 2007). In fact, removal of the serotonin system by an intraventricular infusion of 5,7-dihydroxytryptamine (5,7-DHT), or pharmacological blockade of serotonin neuron activity, by combined activation of 5-HT_{1A} and 5-HT_{1B} receptors, abolishes L-DOPA-induced AIMs in 6-OHDA-lesioned rats. Sub-threshold doses of 5-HT_{1A} and 5-HT_{1B} receptor agonists, which individually have no or very limited effects, are able to produce near-complete suppression of L-DOPA-induced AIMs in L-DOPA-primed 6-OHDA-lesioned rats when administered together (Carta et al., 2007). Moreover, the same drugs were effective in preventing development of AIMs and induction of FosB in striatal projection neurons when chronically administered from the very first dose of L-DOPA (Muñoz et al., 2008).

This effect may be mediated by activity of the 5-HT_1 agonists on autoreceptors located on the cell bodies and terminals of the serotonin neurons, and possibly also by activation of post-synaptic 5-HT_{1A} receptors located in the pre-frontal cortex, which participate to the control of the activity of serotonin neurons via a poly-synaptic feedback loop (Ceci et al., 1994; Hajos et al., 1999).

Inhibition of excessive DA release from serotonin terminals, however, is not the only mechanism that may explain the anti-dyskinetic effect of 5-HT_{1A} and 5-HT_{1B} agonists. Previous studies have shown that activation of post-synaptic 5-HT_{1A} receptors located on the cortico-striatal projection neurons, and their terminals in the striatum, has an inhibitory effect on striatal glutamate release and may therefore contribute to the antidyskinetic effect of 5-HT_{1A} agonists (Antonelli et al., 2005; Mignon and Wolf, 2005; Dupre et

* Corresponding author. Neurobiology Unit, Wallenberg Neuroscience Center, Department of Experimental Medical Science, Lund University, Lund, Sweden.
E-mail address: Manolo.Carta@med.lu.se (M. Carta).
[1] Present address: Experimental Neurology Unit, Department of Neurology, Phillips University, Hans Meerwein Strasse, 350 43 Marburg, Germany.

0014-4886/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2009.05.033



al., 2008). Marin et al. (2009), have recently identified in the subthalamic nucleus an additional area where stimulation of 5-HT_{1A} receptors may produce antidyskinetic effects. As for the 5-HT_{1A} receptors, 5-HT_{1B} also expressed post-synaptically, in striatum and substantia nigra, and their activation has been suggested to provide antidyskinetic effect by inhibiting GABA release (Zhang et al., 2008).

The present study was designed to discriminate between these two alternative modes of action of the 5-HT_1 agonists, i.e. the effect on AIMs mediated by inhibition of DA release from serotonin neurons, and the effect mediated via other, serotonin-independent mechanisms. For this purpose, we studied the effect of increasing doses of the 5-HT_{1A} agonist 8-OH-DPAT and the 5-HT_{1B} agonist CP-94253 (Hjorth and Sharp, 1991; Knobelman et al., 2000), on AIMs induced by either L-DOPA or apomorphine. In contrast to L-DOPA-induced AIMs, which depend on the release of L-DOPA-derived DA from serotonin terminals, the AIMs induced by apomorphine are elicited by a direct action on post-synaptic DA receptors, thus by-passing the serotonin neurons.

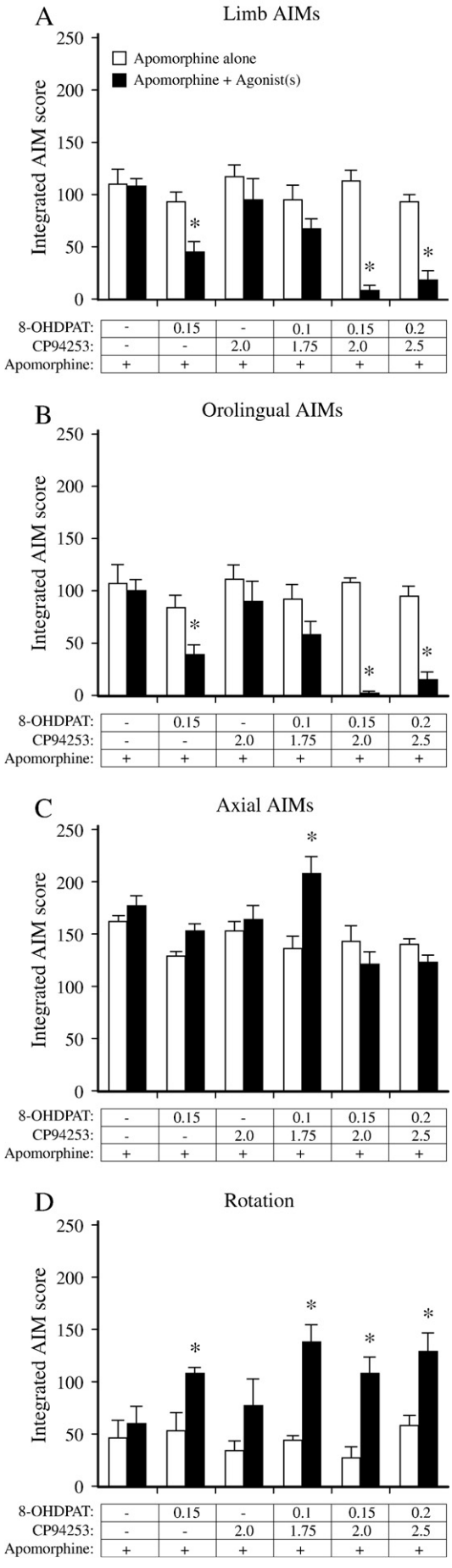
Materials and methods

Experimental design

A total of 120 adult female Sprague–Dawley rats weighing 225–250 g were used in the present study (B&K Universal, Stockholm, Sweden). The animals 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.

At the beginning of the study all animals received a unilateral 6-OHDA lesion in the Medial Forebrain Bundle (MFB), at one site, in order to achieve complete lesion of the nigrostriatal pathway. Three weeks later, the rats were screened behaviorally in the amphetamine-induced rotation test (2.5 mg/kg i.p.). Animals exhibiting ≥6 full body turns/min towards the side of DA deficiency were included in the study. Animals used for the acute experiments (Figs. 1 and 2) were treated with apomorphine (0.05 mg/kg s.c.) or L-DOPA (6 mg/kg i.p., plus 10 mg/kg benserazide) daily for 3–4 weeks, until a stable level of AIMs was achieved. For each experiment, the animals were then allocated into two well-balanced groups (so that the average AIMs score at baseline was equal for the groups used in the same set of experiments), which received either the DA agonist (apomorphine or L-DOPA) alone, or in combination with 5-HT_1 agonists (given s.c. right before L-DOPA/apomorphine) at different doses every 24 h. To avoid possible induction of receptor desensitization due to repeated administration, 5-HT_1 agonist treatment was given to one or the other group in an alternate fashion (so that a 2-day washout was allowed before the same animals were given a second dose of 5-HT_1 agonists). For the chronic study (Fig. 4), animals were allocated into two groups matched on basis of their amphetamine-induced rotation scores. These groups received chronic daily treatment with either apomorphine alone, or in combination with 8-OH-DPAT (0.15 mg/kg) plus CP-94253 (2.0 mg/kg), for 2 weeks. At the end of the treatment, a subgroup of animals was challenged with a threshold dose of apomorphine (0.02 mg/kg) in order to test DA receptor sensitization (in the absence of 5-HT_1 agonist treatment). The remaining animals of the chronic study were sacrificed and brains were used for

Fig. 1. Acute effect of 8-OH-DPAT and CP-94253 on L-DOPA-induced AIMs. Abnormal involuntary movements (AIMs) were monitored after administration of different doses of 8-OH-DPAT and CP-94253 in two groups of 6-OHDA-lesioned dyskinetic rats ($n=6$/group) treated with L-DOPA. Low doses of the compounds were ineffective when tested individually, but highly efficient in reducing all components of AIMs when given in combination. Rotational behavior, by contrast, was marginally affected only at the high-dose combination (values are expressed as mean ± s.e.m., * = $p<0.05$ compared to the L-DOPA-only treated group in the Mann–Whitney test).



immunoistochemical analysis of tyrosine hydroxylase (TH) and FosB expression.

An additional group of 6-OHDA-lesioned rats was primed with daily injections of apomorphine, as above, and then challenged with L-DOPA (Fig. 3). These animals were allocated into two balanced subgroups and subjected to either a serotonin or "sham" lesion to investigate the impact of removing the serotonin innervation on apomorphine-induced AIMs, with or without 5-HT_1 agonist treatment.

6-OHDA lesion

6-OHDA injection was conducted under anesthesia induced by an injectable 20:1 mixture of Fentanyl and Dormitor (Apoteksbolaget, Sweden) using a hamilton syringe attached to a stereotaxic frame (Stoelting, Wood Dale, Illinois). The animals received 6-OHDA (Sigma-Aldrich AB, Sweden) injection into the MFB (14 μg free base in 4 μl) 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.

5,7-DHT injections

Lesions of the serotonergic system were performed under 1–2% isofluorane anesthesia using 5,7-DHT creatine sulfate (10 μg free base in 2 μl 0.02% ascorbate-saline; Sigma-Aldrich AB, Sweden) at the following coordinates, relative to bregma; AP: −3.0 mm; ML: −1.6 mm; DV: −7.8 mm; tooth bar set to −3.3 mm. Injection was performed over 2 min and the needle was kept in place for additional 3 min before retracted. The "sham" controls received similar injection of saline. In order to protect the noradrenergic system, the catecholamine reuptake blocker desipramine (25 mg/kg; Sigma-Aldrich AB, Sweden) was injected i.p. 30 min before 5,7-DHT or saline infusion (Bjorklund et al., 1975). After surgery, all rats received Temgesic (Apoteksbolaget, Sweden) as analgesic treatment and physiological saline to prevent post-surgical dehydration. They were also provided with additional bedding and palatable foods until stabilized intake of food and water were achieved. A two-week recovery period was allowed for the animals.

Behavioral analysis

Apomorphine and L-DOPA-induced AIMs

In all tests AIMs were evaluated according to the rat dyskinesia scale described in detail previously (Lundblad et al., 2002). Briefly, the animals were placed individually in transparent plastic cages without bedding material and scored every 10 or 20 min following the injection of apomorphine or L-DOPA, respectively. Scoring after the apomorphine challenge at the end of the chronic treatment was performed every 5 min. The AIMs were classified into four subtypes according to their topographic distribution as forelimb (Li), orolingual

Fig. 2. Acute effect of 8-OH-DPAT and CP-94253 on apomorphine-induced AIMs. Abnormal involuntary movements (AIMs) were monitored after administration of different doses of 8-OH-DPAT and CP-94253 in two groups of 6-OHDA-lesioned dyskinetic rats ($n=6$/group) treated with apomorphine. The first two bars in each panel represent the baseline of dyskinesia in the two groups. 8-OH-DPAT at 0.15 mg/kg dose was moderately effective when tested individually, while CP-94253 was ineffective at all doses tested. However, combination of the same dose of 8-OH-DPAT with CP-94253 at 2.0 mg/kg dose produced near-complete suppression of limb and orolingual AIMs (A and B). Rotational behavior, by contrast, was markedly increased by agonist administration (D). A higher dose combination did not produce any further reductions (values are expressed as mean ± s.e.m., $*=p<0.05$ compared to the apomorphine-only treated group in the Mann–Whitney test).

(OI) axial (Ax), and locomotive (Lo) dyskinesia (displayed as controlateral 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). The data are presented as integrated scores, area under the curve in a raw data plot of total scores (AIM score multiplied by the interval of observation).

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 (dimensions 40.6 cm×40.6 cm×38.1 cm) 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.

Immunohistochemistry and estimation of FosB-positive cell numbers in striatum

Animals employed in the chronic study (plus saline and agonist-only treated controls) were sacrificed 48 h after the last L-DOPA or L-DOPA + 5-HT_1 agonist injection, the brains were removed and processed for FosB and TH immunohistochemistry (to verify the dopaminergic lesion). Briefly, the brains were cut into 16 μm thickness on a cryostat (HM500M, Microm) and the sections were mounted on plus-charged glass slides (Superfrost+; Electron Microscopy Sciences, PA, USA). Sections were fixed for 30 min in 10% formalin and further rinsed with 3×KPBS + 0.25% Triton-X (KPBS/T). After preincubation for 1 h with 5% NHS (normal horse serum) in KPBS/T, slides were incubated over-night at room temperature with the corresponding primary antibody: FosB (1:15,000; goat polyclonal IgG; SC-48X; Santa Cruz) or TH (1:2000; mouse IgG; Chemicon, MAB 318), followed by 1 h incubation with the corresponding biotinylated secondary antibody (1:250, horse-α-goat, BA9500 or horse-α-mouse, BA2001; Vector Laboratories, Burlingame, CA, USA) and 1 h in avidin–biotin–peroxidase solution (ABC Elite; Vector Laboratories) using 3′,3′-diaminobenzidine as chromogen. Finally, the sections were dehydrated in ascending alcohol solution, cleared in xylene and coverslipped with Depex.

The total number of FosB-positive cells was evaluated by Image J software. Briefly, two high-resolution images were captured, corresponding to the rostral and caudal aspect of the head of striatum (1.00 and −0.30 mm from bregma) using Scanscope GL system with Imagescope v8.2 software. A threshold was set to exclude basal grey matter background staining, and all cells that appeared with higher optical density were automatically calculated by the software to give the total number of cells in the two sections. The number of cells was also obtained for the lateral part of the striatum defined as half the width of the striatum in each section.

HPLC measurements

All animals employed in the acute study were killed and striata rapidly dissected out, frozen on dry ice and stored in a −80 °C freezer until analysis. At the time of the analysis, tissue was homogenized in 0.1 M perchloric acid and centrifuged at 10,000 rpm for 10 min before filtering though minispin filters for additional 3 min at 10,000 rpm. The tissue extracts were then analyzed by HPLC as described earlier (Carta et al., 2006) with minor modifications. Briefly, 25 μl of each sample were injected by a cooled autosampler (Midas) into an ESA Coulochem III coupled with an electrochemical detector. The mobile phase (sodium acetate 5 g/l, Na_2-EDTA 30 mg/l, octane-sulfonic acid 100 mg/l, methanol 10%, pH 4.2) was delivered at a flow rate of 500 μl/min to a reverse phase C18 column (4.6 mm ∅, 150 mm length, Chrompack). The peaks were processed by the Azur Chromatographic Software (Dataly, France).

In agreement with our previous report (Carta et al., 2007), all 6-OHDA-lesioned animals employed in these experiments had near-complete depletion of striatal DA (mean ± s.e.m.: 85.5 ± 4.2 pmol/mg of tissue and 0.6 ± 0.01 pmol/mg of tissue for intact and lesioned side, respectively; $p<0.01$ in the *t*-test). 5,7-DHT lesion, by contrast, produced about 90% reduction in serotonin tissue levels compared to the intact side of the brain (mean ± s.e.m.: 2.5 ± 0.3 pmol/mg of tissue and 0.26 ± 0.02 pmol/mg of tissue for intact and lesioned sides, respectively; $p<0.01$ in the *t*-test).

Statistical analysis

Statistical analysis was performed using SigmaStat Statistical software version 2.0. Group comparisons were performed using the Mann–Whitney test for analysis of dyskinesias. Paired *t*-test and one-way ANOVA followed by Bonferroni post-hoc test were used for analysis of DA and serotonin tissue levels and FosB-positive cell number, respectively.

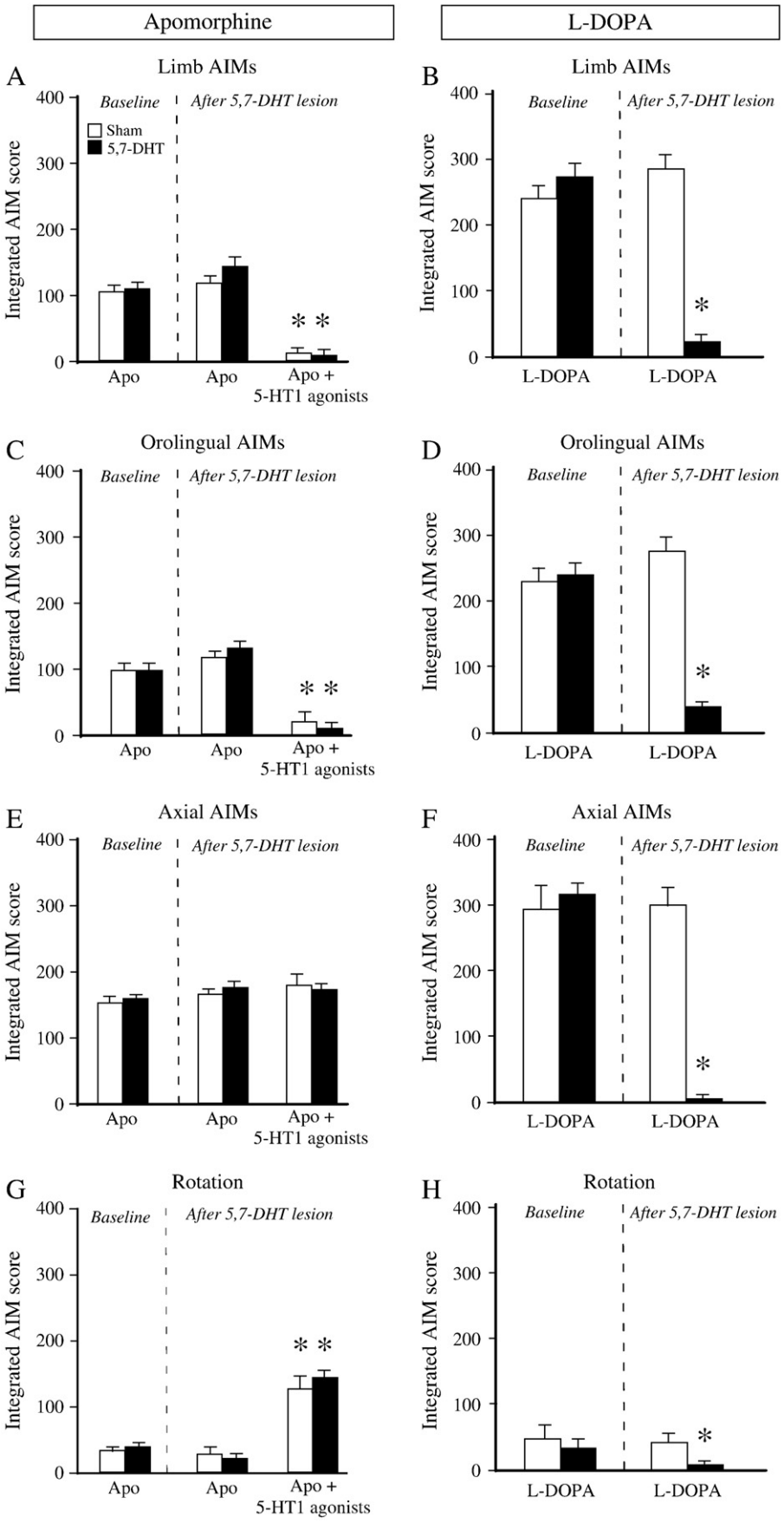
Results

Acute effect of 8-OH-DPAT and CP-94253 on L-DOPA- and apomorphine-induced AIMs

Different doses of 8-OH-DPAT and CP-94253 were tested for their ability to reduce dyskinesia induced by either apomorphine (0.05 mg/kg s.c.) or L-DOPA (6 mg/kg i.p. plus benserazide 10 mg/kg). Two separate groups of 6-OHDA-lesioned, apomorphine- or L-DOPA-primed rats were used for this purpose. Animals were allocated into well-matched subgroups ($n=6$/group), according to their baseline AIMs, which received daily injections of DA agonist (L-DOPA or apomorphine) alone or in combination with the 5-HT_1 agonists 8-OH-DPAT and CP-94253, in doses selected based on our previous report (Carta et al., 2007). As shown in Fig. 1, combination of the two 5-HT_1 agonists was able to significantly decrease L-DOPA-induced AIMs already at low doses (0.05 + 1.0 and 0.1 + 1.75 for 8-OH-DPAT and CP-94253 respectively). At these doses apomorphine-induced AIMs were unaffected (Fig. 2). Significant suppression of apomorphine-induced AIMs was observed at a combination of doses of 0.15 and 2.0 for 8-OH-DPAT and CP-94253, respectively, particularly for the limb and orolingual components, while higher doses were unable to produce further reduction (Fig. 2). Interestingly, and in line with a previous report (Matsubara et al., 2006), the rotational response to apomorphine was potentiated by the combined 5-HT_1 agonist treatment.

Effect of removal of the serotonin innervation on L-DOPA and apomorphine-induced AIMs

An additional group of rats ($n=16$) was primed with daily injections of apomorphine until a stable level of AIMs was achieved. A single challenge with L-DOPA was also performed and AIMs were scored. Animals were then allocated into two well-balanced groups ($n=8$), which received either an injection of 5,7-DHT (10 μg in 2 μl of free base) into the MFB, or a sham lesion, in order to study the impact of removing the serotonin innervation on AIMs expression. In line with our previous report (Carta et al., 2007), AIMs induced by L-DOPA were reduced by more than 90% following the 5,7-DHT lesion, and all components of AIMs were equally affected (Figs. 3B, D, F and H). Apomorphine-induced AIMs, by contrast, remained unaltered (Figs. 3A, C, E and G). Removal of the serotonin innervation and consequent reduction of the serotonin tone appear therefore not to affect apomorphine-induced AIMs. Interestingly, the inhibiting effect of 5-HT_1 agonists on apomorphine-induced AIMs (0.15 mg/kg 8-OH-DPAT plus 2.0 mg/kg CP-94253) was unchanged by the 5,7-DHT lesion.


Apomorphine
L-DOPA
A
Limb AIMs
Baseline
After 5,7-DHT lesion
Sham
5,7-DHT
Integrated AIM score
400
300
200
100
0
* *
Apo
Apo
Apo +
5-HT1 agonists
B
Limb AIMs
Baseline
After 5,7-DHT lesion
Integrated AIM score
*
L-DOPA
L-DOPA
C
Orolingual AIMs
Baseline
After 5,7-DHT lesion
Integrated AIM score
* *
Apo
Apo
Apo +
5-HT1 agonists
D
Orolingual AIMs
Baseline
After 5,7-DHT lesion
Integrated AIM score
*
L-DOPA
L-DOPA
E
Axial AIMs
Baseline
After 5,7-DHT lesion
Integrated AIM score
Apo
Apo
Apo +
5-HT1 agonists
F
Axial AIMs
Baseline
After 5,7-DHT lesion
Integrated AIM score
*
L-DOPA
L-DOPA
G
Rotation
Baseline
After 5,7-DHT lesion
Integrated AIM score
* *
Apo
Apo
Apo +
5-HT1 agonists
H
Rotation
Baseline
After 5,7-DHT lesion
Integrated AIM score
*
L-DOPA
L-DOPA

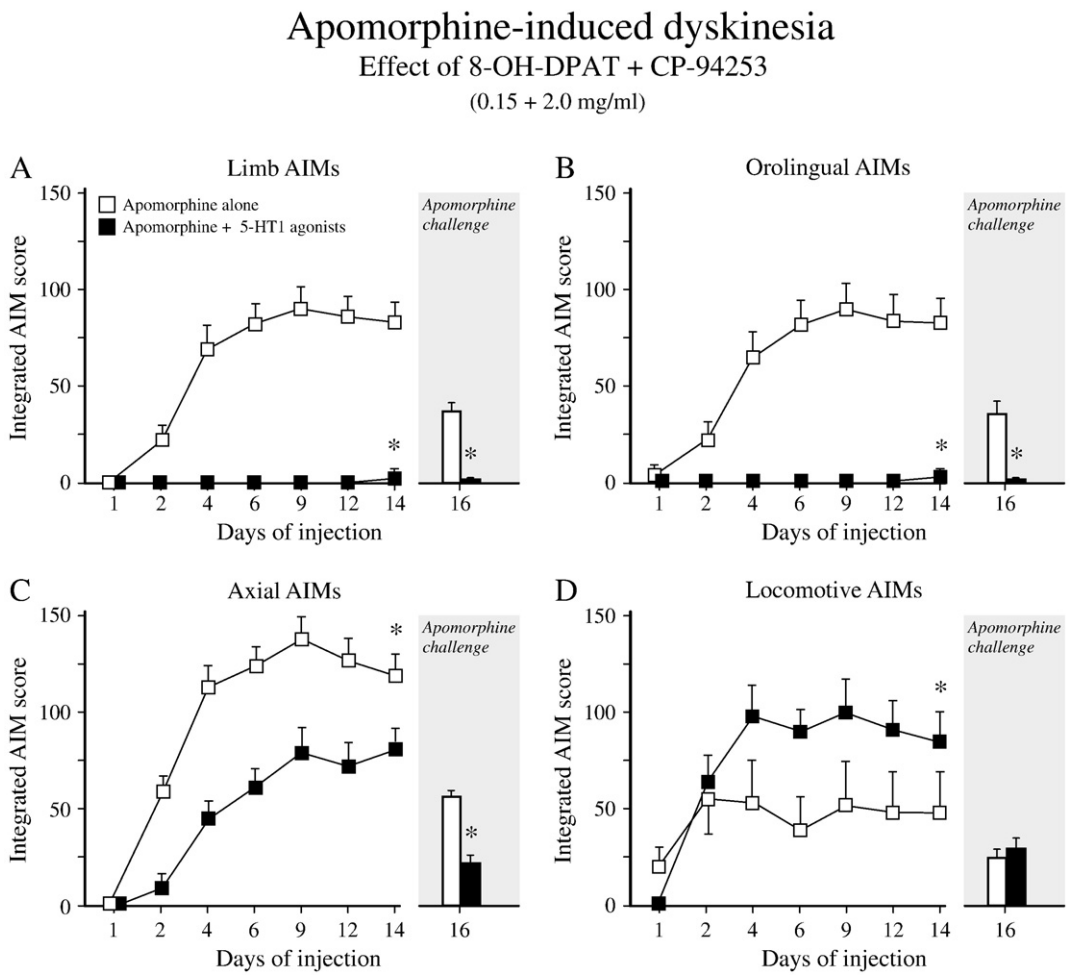


Fig. 4. Chronic effects of 8-OH-DPAT and CP-94253 on apomorphine-induced AIMs. The ability of 8-OH-DPAT and CP-94253 (at 0.15 and 2.0 mg/kg dose respectively) to prevent development of AIMs induced by daily treatment with apomorphine (0.05 mg/kg) was tested in another group of rats ($n = 10$/group). The results show that combination of the two 5-HT_1 agonists completely prevented appearance of limb and orolingual AIMs (A and B), whereas the axial component was less, but significantly reduced (C). Apomorphine-induced rotation, by contrast, was potentiated by the 5-HT_1 agonist treatment (D). A challenge with a low dose of apomorphine (0.02 mg/kg), performed at the end of the treatment period in the absence of 5-HT_1 agonists, revealed less DA receptor sensitization in the 5-HT_1 agonist-treated group (values are expressed as mean ± s.e.m., * = $p < 0.05$ in the Mann–Whitney test).

Chronic effect of 8-OH-DPAT and CP-94253 on development of apomorphine-induced AIMs

We have recently shown that sub-threshold doses of 5-HT_{1A} and 5-HT_{1B} agonists in combination (0.05 and 1.0 for 8-OH-DPAT and CP-94253, respectively) can prevent development of L-DOPA-induced AIMs when administered daily together with the indirect DA agonist (Muñoz et al., 2008). Here, we asked whether combination of 5-HT_1 agonists, at the doses found to be effective in acutely dampening apomorphine-induced AIMs, may also be efficacious in blocking the development of AIMs induced by daily treatment with apomorphine.

Two groups of drug-naïve 6-OHDA-lesioned rats ($n = 10$/group) were treated with either apomorphine (0.05 mg/kg s.c.) alone, or in combination with the two 5-HT_1 receptor agonists, at doses that were found to be efficacious in the acute tests (0.15 and 2.0 mg/kg s.c. for 8-OH-DPAT and CP-94253, respectively). As shown in Figs. 4A and B, 8-OH-DPAT and CP-94253 in combination were able to completely prevent appearance of limb and orolingual AIMs after 2 weeks of treatment. The axial AIMs were also significantly reduced, although to a lesser extent (Fig. 4C). In line with the results obtained in the acute experiment, 8-OH-DPAT and CP-94253 appeared to potentiate the rotational behavior induced by apomorphine (Fig. 4D). A similar effect was seen on horizontal activity in the open-field test (Fig. 5A).

To unveil a possible masking, rather than inhibiting, effect of the 5-HT_1 agonists on the expression of AIMs, a subgroup of animals ($n = 4$/group) was challenged at the end of the chronic study with a low dose of apomorphine (0.02 mg/kg) in the absence of 5-HT_1 agonist treatment. As shown in Fig. 4 (day 16), the results indicate lower DA receptor sensitization in the 5-HT_1 agonist-treated group compared with the apomorphine-only treated group.

Effect of 8-OH-DPAT and CP-94253 on apomorphine-induced FosB upregulation

A subgroup of animals employed in the chronic study ($n = 6$/group) was analyzed for induction of FosB (together with saline and

Fig. 3. Effect of 5,7-DHT lesions on AIMs induced by L-DOPA or apomorphine, and on the antidyskinetic efficacy of 5-HT_1 agonists. Two groups of 6-OHDA-lesioned rats ($n = 8$/group) were primed with apomorphine and allocated to two well-balanced subgroups, which also received a challenge with L-DOPA. Animals were then subjected to either a serotonin lesion by the selective serotonin toxin 5,7-DHT, or to a sham lesion, in order to investigate the impact of removal of the serotonin innervation on AIMs. The serotonin lesion produced near-complete suppression of L-DOPA-induced AIMs (B, D, F, H), while apomorphine-induced AIMs remained unaffected (A, C, E, G). Importantly, the inhibitory effect of 5-HT_1 agonists (at 0.15 plus 2.0 mg/kg dose of 8-OH-DPAT and CP-94253, respectively) on apomorphine-induced AIMs was unaltered by the 5,7-DHT lesion (values are expressed as mean ± s.e.m., * = $p < 0.05$ in the Mann–Whitney test).

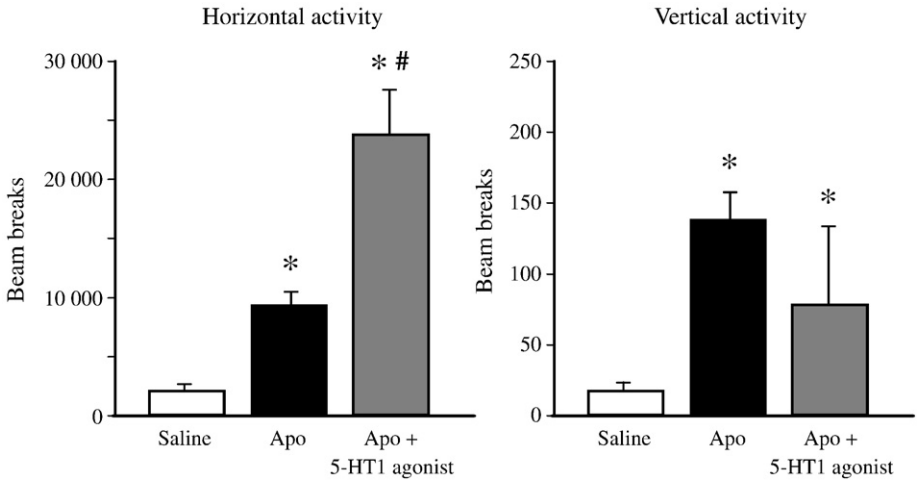


Fig. 5. Effect of 5-HT_1 agonists on apomorphine-induced horizontal and vertical activities. Horizontal and vertical activities were measured in open-field chambers using animals employed in the chronic study. The results show that treatment with 5-HT_1 agonists did not reduce apomorphine-induced motor activation but rather increased its effect on horizontal activity (values are expressed as mean ± s.e.m.; $p<0.05$ in the Kruskal–Wallis test followed by Student–Newman Keuls; * = different from saline; # = different from apomorphine only).

5-HT_1 agonist-only treated groups as controls), a well-established marker of striatal DA receptor sensitization (Andersson et al., 1999). The results showed that apomorphine treatment induced a significant upregulation in the number of striatal FosB-positive cells (Fig. 6). However, no significant difference was found between apomorphine-only and apomorphine plus 5-HT_1 agonist-treated groups, neither in the whole striatum (mean ± s.e.m.: 3317 ± 371, 2626 ± 564, 1739 ± 372, 1517 ± 367 for apomorphine-only, apomorphine + 5-HT_1 agonists, 5-HT_1 agonist-only and saline respectively) nor in the lateral part (mean ± s.e.m.: 2000 ± 203, 1477 ± 304, 635 ±

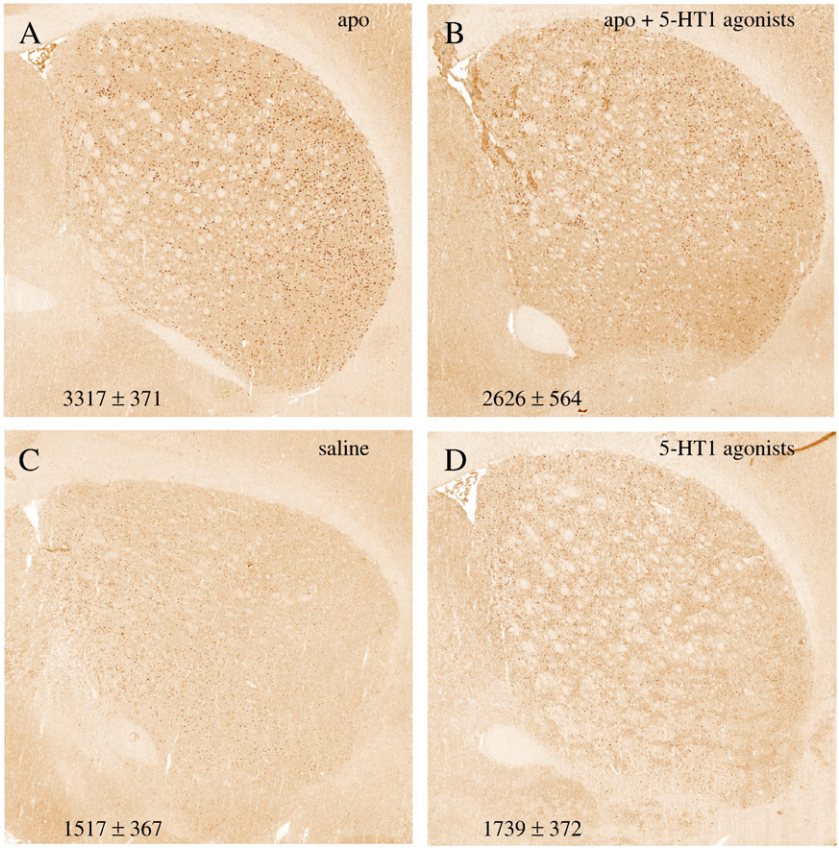


Fig. 6. Effect of 8-OH-DPAT and CP-94253 on apomorphine-induced FosB expression. A subgroup of animals employed in the chronic study ($n = 6$/group), together with saline and 5-HT_1 agonist-only treated groups ($n = 5$/group), were used for investigating treatment-induction of FosB expression in the striatum. Significant upregulation of FosB was found in the apomorphine-treated groups compared to saline and 5-HT_1 agonist-only treated animals. No significant difference was found between groups treated with apomorphine alone or apomorphine plus 5-HT_1 agonists in the number of FosB-positive cells, neither in the whole striatum (mean ± s.e.m.: 3317 ± 371, 2626 ± 564, 1739 ± 372, 1517 ± 367 for apomorphine-only, apomorphine + 5-HT_1 agonists, 5-HT_1 agonist-only and saline respectively) nor in the lateral part (mean ± s.e.m.: 2000 ± 203, 1477 ± 304, 635 ± 178, 520 ± 207 for apomorphine-only, apomorphine + 5-HT_1 agonists, 5-HT_1 agonist-only and saline respectively). $p<0.05$ for apomorphine and apomorphine + 5-HT_1 agonists vs agonist-only and saline, ANOVA followed by Bonferroni. Values for the whole striatum are given in the figure.

178, 520 ± 207 for apomorphine-only, apomorphine + 5-HT$_1$ agonists, 5-HT$_1$ agonist-only and saline respectively), which has been identified as a critical area involved in the induction of L-DOPA-induced AIMs.

Discussion

In the present study, we evaluated the ability of increasing doses of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonists to suppress L-DOPA- and apomorphine-induced AIMs in the rat 6-OHDA model. This comparison allowed us to distinguish between two different mechanisms accounting for the antidyskinetic effect induced by the 5-HT$_1$ agonist, i.e. one dependent on the activity of the serotonin neurons, the other one mediated by a direct action on post-synaptic targets. We found that combination of 8-OH-DPAT and CP-94253 was able to provide complete suppression of L-DOPA-induced AIMs already at sub-threshold doses (i.e. doses that do not provide any reduction of L-DOPA-induced AIMs when given individually). At these doses the agonists are likely to act by dampening release of L-DOPA-derived DA from the serotonin neurons (Carta et al., 2007). In support of this view, low doses of 5-HT$_1$ agonists were ineffective against apomorphine-induced AIMs and removal of the serotonin innervation suppressed AIMs induced by L-DOPA, but not those induced by apomorphine. Furthermore, Kannari et al. (2001), have shown that administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT can produce a near-complete suppression of L-DOPA-derived striatal DA release in parkinsonian rats.

It is known that suppression of activity of serotonin neurons can be achieved by activation of pre-synaptic 5-HT$_{1A}$ and 5-HT$_{1B}$ autoreceptors, located at the soma and terminal levels, respectively. In addition, cortical post-synaptic 5-HT$_{1A}$ receptors have been shown to contribute to the control of firing rate of serotonin neurons via a poly-synaptic feedback loop (Ceci et al., 1994; Hajos et al., 1999). The suppression of apomorphine-induced AIMs, which was seen at higher doses of the 5-HT$_1$ agonists, is most likely mediated by activation of post-synaptic 5-HT$_1$ receptors. The use of apomorphine, in fact, allowed us to by-pass the serotonin neurons, and circumvent the antidyskinetic effect supposedly mediated by inhibiting DA release from serotonin terminals. Activation of post-synaptic 5-HT$_{1A}$ receptors located in cortical areas has been proposed to contribute to the antidyskinetic effect of 5-HT$_{1A}$ agonists by reducing striatal glutamate release and thus dampening the over-activation of striatal neurons (Antonelli et al., 2005; Mignon and Wolf, 2005). Furthermore, it has been recently demonstrated that striatal 5-HT$_{1A}$ receptors can contribute to the control of glutamate release by acting at pre-synaptic receptors expressed on glutamatergic terminals (Dupre et al., 2008). These authors have shown that 8-OH-DPAT can reduce AIMs induced by the DA D$_1$ receptor agonist SKF81297 not only when administered systemically, but also when directly infused into the striatum of dyskinetic rats. The antidyskinetic effect induced by reduction of striatal glutamate release is in agreement with previous reports showing that glutamate receptor antagonists, such as the NMDA antagonist amantadine, can provide significant reduction of dyskinesia in both animal models and PD (Parkinson´s disease) patients (Crosby et al., 2003; Dekundy et al., 2007).

Besides their role as pre-synaptic receptors regulating serotonin terminal release, 5-HT$_{1B}$ receptors are also expressed as heteroreceptors on post-synaptic neurons. Activation of these receptors at the striatal level has been suggested to reduce local release of GABA, which may further contribute to the antidyskinetic effect of 5-HT$_{1B}$ agonists (Zhang et al., 2008). In the present study, the 5-HT$_{1B}$ receptor agonist CP-94253 did not induce a significant antidyskinetic effect when given individually at the doses tested, neither on L-DOPA nor on apomorphine-induced AIMs. However, we showed for the first time that CP-94253 was able to potentiate the antidyskinetic effect of 8-OH-DPAT on AIMs induced by either drug. In line with our previous report (Carta et al., 2007), 5-HT$_{1A}$ and 5-HT$_{1B}$ agonists appear to act synergistically in reducing L-DOPA-induced AIMs already at very low doses. Similarly, a greater-than-addictive effect was observed also on apomorphine-induced AIMs when the agonists were used at higher doses. However, this effect was limited to limb and orolingual AIMs and the underlying mechanism appears to be different to that involved in reduction of L-DOPA-induced AIMs. The case for a different mechanism of action of CP-94253 on apomorphine-induced AIMs with respect to the one underlying the effect of 8-OH-DPAT is supported by the fact that doses higher than 0.15 mg/kg of 8-OH-DPAT did not further increase the antidyskinetic efficacy of the drug (not shown), while a further reduction was induced by adding CP-94253.

One may argue that full suppression of serotonin release, likely achieved by high doses of 5-HT$_1$ agonists, could also play a role in the effect of 8-OH-DPAT and CP-94253 on apomorphine-induced AIMs, given the ability of serotonin to modulate DA transmission; however, this seems unlikely, as removal of the serotonin innervation by 5,7-DHT neither affected apomorphine-induced AIMs nor the antidyskinetic efficacy of high doses of 5-HT$_1$ agonists, as seen in a separate group of 6-OHDA-lesioned apomorphine-treated rats (see Fig. 3). This important finding reveals that such an antidyskinetic effect is independent on activation of pre-synaptic 5-HT$_1$ receptors and inhibition of serotonin neuron activity.

Interestingly, the effect of the 5-HT$_1$ agonists on apomorphine-induced rotation was distinctly different from that on limb and orolingual AIMs in either the acute or chronic study (i.e. limb and orolingual AIMs were suppressed, while the rotational response was markedly increased), suggesting that these two actions of the drug are mediated by different cellular mechanisms.

The significance of rotational behavior induced by L-DOPA or direct DA agonists has been the subject of much debate. While some authors have viewed rotational behavior as a maladaptive response to dopaminergic drugs, and a possible model for L-DOPA- and apomorphine-induced AIMs, others have considered that it reflects the therapeutic efficacy of DA receptor active compounds [see Cenci et al. (2002) and Marin et al. (2006) for recent reviews]. In this context, our observation that 5-HT$_1$ agonists have opposite effects on AIMs and rotation, is particularly interesting. A similar dissociation between AIMs and rotation was recently reported by Dupre and colleagues (2007, 2008), who observed decreased AIMs but increased rotation induced by the DA D$_1$ receptor agonist SKF81297 when co-administered with 8-OH-DPAT, as compared to animals treated with SKF81297 alone. This suggests that the effect seen in our study may be due to an interaction between D$_1$ and 5-HT$_{1A}$ receptors. In support of an antiparkinsonian interpretation of the increased apomorphine-induced rotational response observed after 5-HT$_1$ agonist administration, DA D$_2$ receptor agonists, such as bromocriptine and ropinirole, are known to provoke intensive contraversive rotation in 6-OHDA-lesioned rats, without inducing AIMs (Cenci et al., 2002; Ravenscroft et al., 2004). Overall these results point to a different biological significance of drug-induced rotation and drug-induced AIMs in the rat 6-OHDA model.

Induction of FosB expression

The present study is also the first to demonstrate the ability of 5-HT$_1$ agonists to prevent development of limb and orolingual AIMs induced by chronic treatment with apomorphine. Appearance of dyskinesia has been associated with upregulation of markers such as FosB in striatal neurons (Andersson et al., 1999). While significantly lower numbers of FosB-positive cells would be expected in the non-dyskinetic animals, no significant difference was found here between the apomorphine-treated groups, with or without 5-HT$_1$ agonist treatment employed in the chronic study. Nevertheless, the 5-HT$_1$ agonists appear to provide a real protection rather than a masking effect, since a challenge with a low dose of apomorphine (given at the end of the chronic treatment, in the absence of 5-HT$_1$ agonists),

triggered significantly less AIMs in the 5-HT$_1$ agonists group, suggesting that DA receptors were less sensitized in those rats. The lack of a significant effect of agonist treatment on FosB expression may reflect the increased rotational response induced by 5-HT$_1$ agonists in the apomorphine-treated animals. In support of this interpretation, we have recently observed high numbers of FosB-positive cells in a non-dyskinetic rat that developed strong rotation in response to L-DOPA treatment (Muñoz et al., 2008).

Side effects induced by 5-HT$_1$ agonist treatment

Our results support the case for the use of 5-HT$_1$ agonists to counteract dyskinesia in PD patients. Use of 5-HT$_1$ agonists as antidyskinetic agents, however, is not without complications. In rodents, stimulation of 5-HT$_{1A}$ receptors is known to induce components of the so-called serotonin syndrome. This syndrome, which is characterized by flat body posture, reciprocal forepaw treading, head weaving and lower lip retraction, is seen in rats treated with 5-HT$_{1A}$ agonists, including 8-OH-DPAT (Goodwin et al., 1986; Smith and Peroutka, 1986; Yamada et al., 1988; Hoyer et al., 2002; Carey et al., 2004). In humans, the side effects reported after use of serotonergic drugs or MAO inhibitors are fever, confusion, agitation, ataxia (Sporer, 1995). At low doses of the 5-HT$_1$ agonists (0.05 plus 2.0 mg/kg for 8-OH-DPAT and CP-94253, respectively), which produced near-complete suppression of L-DOPA-induced AIMs, we did not observe any sign of serotonin syndrome at any time-point (Carta et al., 2007; Muñoz et al., 2008). The high-dose combination, by contrast, induced components of the serotonin syndrome, particularly flat body posture and lower lip retraction, in the majority of animals. This effect was most likely due to 5-HT$_{1A}$ stimulation, as it was seen when 8-OH-DPAT, but not CP-94253, was given individually at the higher dose (Carta et al., 2007). This is in line with the view that the serotonin syndrome is induced by post-synaptic 5-HT$_{1A}$ receptor stimulation, as suggested previously (Goodwin et al., 1986; Yamada et al., 1988; Sporer, 1995; Carey et al., 2004). Apomorphine, when co-administered with the high-dose combination of the two 5-HT$_1$ agonists, appeared to partly counteract this side effect due to the motor stimulatory effect of the dopaminergic drug. This observation underscores the importance of direct, visual observations of the drug-treated animals' behavior as a supplement to instrumental measurements (see O'Neill and Parameswaran, 1997). Possible appearance of serotonin syndrome should therefore be taken into consideration when manipulating serotonin neurotransmission by 5-HT receptor agonists.

Clinical implications

The 5-HT$_{1A}$ partial agonist Sarizotan has already been tested for its ability to suppress dyskinesia in L-DOPA-treated dyskinetic patients (Olanow et al., 2004; Bara-Jimenez et al., 2005; Goetz et al., 2007). Despite initial promising reports, however, a phase III trial has been recently terminated for lack of efficacy (see Merck web site at http://media.merck.de). While this trial was performed with a low dose of the drug, a previous phase II investigation had explored also higher doses. The antidyskinetic effect of the drug was in that case accompanied by worsening of the PD score and other side effects (Olanow et al., 2004). This is in line with our own data showing that targeting of the 5-HT$_{1A}$ receptors alone provides a significant antidyskinetic effect only at higher doses, i.e. at doses that activate both pre- and post-synaptic receptors, and is associated with an increased risk of adverse side effects (Iravani et al., 2006). Simultaneous activation of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, by contrast, is effective in dampening L-DOPA-induced AIMs already at low doses, in the absence of side effects. In support of this approach we have recently shown that sub-threshold doses of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonists, in combination, can provide near-complete suppression of L-DOPA-induced AIMs also in MPTP-treated macaques, without signs of side effects or interference with the antiparkinsonian efficacy of L-DOPA (Muñoz et al., 2008). Combinations employing higher doses of the 5-HT$_{1A}$ agonist, by contrast, were associated with a partial worsening of the therapeutic efficacy of L-DOPA. Despite the negative outcome of the recent Sarizotan trial, therefore, serotonin agonists hold great promise as antidyskinetic agents in PD patients. Further development of this approach, however, should focus on low-dose treatments with drugs combining action on both 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors.

In summary, the results of the present study are relevant for the understanding of the mechanisms underlying the antidyskinetic effect of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonists. While both serotonin neuron-dependent and -independent mechanisms of action have been already proposed, by our group and others, groups to contribute to the antidyskinetic effect of 5-HT$_1$ agonists, no previous study has been designed to evaluate their relative contribution. Thus, we found that inhibition of L-DOPA-induced AIMs, by combination of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor agonists, takes place at lower doses compared to the ones required to suppress apomorphine-induced AIMs. We proposed that the mechanism underlying the effect of low doses of 5-HT$_1$ agonists on L-DOPA-induced AIMs relies on inhibition of serotonin-mediated dopamine release, while the effect of higher doses of the agonists on apomorphine-induced AIMs is triggered by serotonin neuron-independent mechanisms such as activation of post-synaptic 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, conceivably leading to a reduction in striatal glutamate and GABA release. In support of this view, we found that 5,7-DHT lesions suppressed AIMs induced by L-DOPA, but neither affected apomorphine-induced AIMs, nor the antidyskinetic effect of higher doses of the agonists, supporting the case for an autoreceptor independent mechanism of action of these compounds on apomorphine-induced AIMs. The appearance of side effects, such as serotonin syndrome components, at high doses of 5-HT$_{1A}$ agonists underscores the importance of the synergistic effect between 5-HT$_{1A}$ and 5-HT$_{1B}$ agonists (which allows complete suppression of L-DOPA-induced dyskinesia already at sub-threshold doses) as the basis for low-dose combination of these compounds for the treatment L-DOPA-induced dyskinesia in PD patients.

Acknowledgments

We thank Ulla Jarl, Anneli Josefsson and Bengt Mattsson, for expert technical assistance. This study was supported by grants from the Swedish Research Council (04X-3874 to AB) and from Parkinsonfonden. Manolo Carta was supported by the Parkinson's Disease Foundation and Michael J Fox Foundation.

References

Andersson, M., Hilbertson, A., Cenci, M.A., 1999. Striatal fosB expression is causally linked with L-DOPA-induced abnormal involuntary movements and the associated upregulation of striatal prodynorphin mRNA in a rat model of Parkinson's disease. Neurobiol. Dis. 6, 461–474.

Antonelli, T., Fuxe, K., Tomasini, M.C., Bartoszyk, G.D., Seyfried, C.A., Tanganelli, S., Ferraro, L., 2005. Effects of Sarizotan on the corticostriatal glutamate pathways. Synapse 58, 193–199.

Bara-Jimenez, W., Bibbiani, F., Morris, M.J., Dimitrova, T., Sherzai, A., Mouradian, M.M., Chase, T.N., 2005. Effects of serotonin 5-HT1A agonist in advanced Parkinson's disease. Mov. Disord. 20, 932–936.

Bjorklund, A., Baumgarten, H.G., Rensch, A., 1975. 5,7-Dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. J. Neurochem. 24, 833–835.

Carey, R.J., Depalma, G., Damianopoulos, E., Muller, C.P., Huston, J.P., 2004. The 5-HT1A receptor and behavioral stimulation in the rat: effects of 8-OHDPAT on spontaneous and cocaine-induced behavior. Psychopharmacology (Berl) 177, 46–54.

Carta, M., Stancampiano, R., Tronci, E., Collu, M., Usiello, A., Morelli, M., Fadda, F., 2006. Vitamin A deficiency induces motor impairments and striatal cholinergic dysfunction in rats. Neuroscience 139, 1163–1172.

Carta, M., Carlsson, T., Kirik, D., Bjorklund, A., 2007. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain 130, 1819–1833.

Ceci, A., Baschirotto, A., Borsini, F., 1994. The inhibitory effect of 8-OH-DPAT on the firing

activity of dorsal raphe serotoninergic neurons in rats is attenuated by lesion of the frontal cortex. Neuropharmacology 33, 709–713.
Cenci, M.A., Whishaw, I.Q., Schallert, T., 2002. Animal models of neurological deficits: how relevant is the rat? Nat. Rev., Neurosci. 3, 574–579.
Crosby, N.J., Deane, K.H., Clarke, C.E., 2003. Amantadine for dyskinesia in Parkinson's disease. Cochrane Database Syst. Rev. CD003467.
Dekundy, A., Lundblad, M., Danysz, W., Cenci, M.A., 2007. Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. Behav. Brain Res. 179, 76–89.
Dupre, K.B., Eskow, K.L., Negron, G., Bishop, C., 2007. The differential effects of 5-HT(1A) receptor stimulation on dopamine receptor-mediated abnormal involuntary movements and rotations in the primed hemiparkinsonian rat. Brain Res. 1158, 135–143.
Dupre, K.B., Eskow, K.L., Barnum, C.J., Bishop, C., 2008. Striatal 5-HT(1A) receptor stimulation reduces D1 receptor-induced dyskinesia and improves movement in the hemiparkinsonian rat. Neuropharmacology 55, 1321–1328.
Goetz, C.G., Damier, P., Hicking, C., Laska, E., Muller, T., Olanow, C.W., Rascol, O., Russ, H., 2007. Sarizotan as a treatment for dyskinesias in Parkinson's disease: a double-blind placebo-controlled trial. Mov. Disord. 22, 179–186.
Goodwin, G.M., De Souza, R.J., Wood, A.J., Green, A.R., 1986. The enhancement by lithium of the 5-HT1A mediated serotonin syndrome produced by 8-OH-DPAT in the rat: evidence for a post-synaptic mechanism. Psychopharmacology (Berl). 90, 488–493.
Hajos, M., Hajos-Korcsok, E., Sharp, T., 1999. Role of the medial prefrontal cortex in 5-HT1A receptor-induced inhibition of 5-HT neuronal activity in the rat. Br. J. Pharmacol. 126, 1741–1750.
Hjorth, S., Sharp, T., 1991. Effect of the 5-HT1A receptor agonist 8-OH-DPAT on the release of 5-HT in dorsal and median raphe-innervated rat brain regions as measured by in vivo microdialysis. Life Sci. 48, 1779–1786.
Hoyer, D., Hannon, J.P., Martin, G.R., 2002. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol. Biochem. Behav. 71, 533–554.
Iravani, M.M., Tayarani-Binazir, K., Chu, W.B., Jackson, M.J., Jenner, P., 2006. In MPTP treated primates, the selective 5-HT1a agonist (R)-(+)-8-hydroxy-DPAT inhibits levodopa-induced dyskinesia but only with increased motor disability. J. Pharmacol. Exp. Ther. 319, 1225–1234.
Kannari, K., Tanaka, H., Maeda, T., Tomiyama, M., Suda, T., Matsunaga, M., 2000. Reserpine pretreatment prevents increases in extracellular striatal dopamine following L-DOPA administration in rats with nigrostriatal denervation. J. Neurochem. 74, 263–269.
Kannari, K., Yamato, H., Shen, H., Tomiyama, M., Suda, T., Matsunaga, M., 2001. Activation of 5-HT(1A) but not 5-HT(1B) receptors attenuates an increase in extracellular dopamine derived from exogenously administered L-DOPA in the striatum with nigrostriatal denervation. J. Neurochem. 76, 1346–1353.
Knobelman, D.A., Kung, H.F., Lucki, I., 2000. Regulation of extracellular concentrations of 5-hydroxytryptamine (5-HT) in mouse striatum by 5-HT1A and 5-HT1B receptors. J. Phar. Exp. Ther. 292, 1111–1117.
Lundblad, M., Andersson, M., Winkler, C., Kirik, D., Wierup, N., Cenci, M.A., 2002. Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. Eur. J. Neurosci. 15, 120–132.
Marin, C., Rodriguez-Oroz, M.C., Obeso, J.A., 2006. Motor complications in Parkinson's disease and the clinical significance of rotational behavior in the rat: have we wasted our time? Exp. Neurol. 197, 269–274.
Marin, C., Aguilar, E., Rodriguez-Oroz, M.C., Bartoszyk, G.D., Obeso, J.A., 2009. Local administration of Sarizotan into the subthalamic nucleus attenuates levodopa-induced dyskinesias in 6-OHDA-lesioned rats. Psychopharmacology. doi:10.1007/s00213-00801452-9.
Matsubara, K., Shimizu, K., Suno, M., Ogawa, K., Awaya, T., Yamada, T., Noda, T., Satomi, M., Ohtaki, K., Chiba, K., Tasaki, Y., Shiono, H., 2006. Tandospirone, a 5-HT1A agonist, ameliorates movement disorder via non-dopaminergic systems in rats with unilateral 6-hydroxydopamine-generated lesions. Brain Res. 1112, 126–133.
Mignon, L.J., Wolf, W.A., 2005. 8-hydroxy-2-(di-n-propylamino)tetralin reduces striatal glutamate in an animal model of Parkinson's disease. NeuroReport 16, 699–703.
Muñoz, A., Li, Q., Gardoni, F., Marcello, E., Qin, C., Carlsson, T., Kirik, D., Di Luca, M., Björklund, A., Bezard, E., Carta, M., 2008. Combined 5-HT1A and 5-HT1B receptor agonists for the treatment of L-DOPA-induced dyskinesia. Brain 131, 3380–3394.
O'Neill, M.F., Parameswaran, T., 1997. RU24969-induced behavioural syndrome requires activation of both 5HT1A and 5HT1B receptors. Psychopharmacology (Berl) 132, 255–260.
Olanow, C.W., Damier, P., Goetz, C.G., Mueller, T., Nutt, J., Rascol, O., Serbanescu, A., Deckers, F., Russ, H., 2004. Multicenter, open-label, trial of Sarizotan in Parkinson disease patients with levodopa-induced dyskinesias (the SPLENDID Study). Clin. Neuropharmacol. 27, 58–62.
Paxinos, G., Watson, C., 1998. The Rat Brain in Stereotaxic Coordinates. Academic Press, San Diego.
Ravenscroft, P., Chalon, S., Brotchie, J.M., Crossman, A.R., 2004. Ropinirole versus L-DOPA effects on striatal opioid peptide precursors in a rodent model of Parkinson's disease: implications for dyskinesia. Exp. Neurol. 185, 36–46.
Smith, L.M., Peroutka, S.J., 1986. Differential effects of 5-hydroxytryptamine1a selective drugs on the 5-HT behavioral syndrome. Pharmacol. Biochem. Behav. 24, 1513–1519.
Sporer, K.A., 1995. The serotonin syndrome. Implicated drugs, pathophysiology and management. Drug Saf. 13, 94–104.
Tanaka, H., Kannari, K., Maeda, T., Tomiyama, M., Suda, T., Matsunaga, M., 1999. Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. NeuroReport 10, 631–634.
Yamada, J., Sugimoto, Y., Horisaka, K., 1988. The behavioural effects of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in mice. Eur. J. Pharmacol. 154, 299–304.
Zhang, X., Andren, P.E., Greengard, P., Svenningsson, P., 2008. Evidence for a role of the 5-HT1B receptor and its adaptor protein, p11, in L-DOPA treatment of an animal model of Parkinsonism. Proc. Natl. Acad. Sci. U.S.A. 105, 2163–2168.