Neurobiology of Disease 39 (2010) 352–361

Contents lists available at ScienceDirect

Neurobiology of Disease

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

A mGluR5 antagonist under clinical development improves L-DOPA-induced dyskinesia in parkinsonian rats and monkeys

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ARTICLE INFO

Article history:
Received 9 March 2010
Revised 28 April 2010
Accepted 1 May 2010
Available online 7 May 2010

Keywords:
Dopamine replacement
Motor complication
Rotarod
Rotation
AIMs
Involuntary movements
Dopamine denervation
mGluR
Glutamate receptor
Neurotransmitter
Striatum
LID

ABSTRACT

L-DOPA remains the gold-standard treatment for Parkinson's disease but causes motor fluctuations and dyskinesia. Metabotropic glutamate receptor type 5 (mGluR5) has been proposed as a target for antidyskinetic therapies. Here, we evaluate the effects of fenobam, a noncompetitive mGluR5 antagonist already tested in humans, using rodent and nonhuman primate models of Parkinson's disease. In both animal models, acute administration of fenobam attenuated the L-DOPA-induced abnormal involuntary movements (50–70% reduction at the doses of 30 mg/kg in rats and 10 mg/kg in monkeys). The effect consisted in a reduction of peak-dose dyskinesia, whereas the end-dose phase was not affected. Chronic administration of fenobam to previously drug-naïve animals (de novo treatment) attenuated the development of peak-dose dyskinesia without compromising the anti-parkinsonian effect of L-DOPA. In addition, fenobam prolonged the motor stimulant effect of L-DOPA. We conclude that fenobam acts similarly in rat and primate models of L-DOPA-induced dyskinesia and represents a good candidate for antidyskinetic treatment in Parkinson's disease.

Introduction

The striatum is densely innervated by dopaminergic (DA) fibers from the substantia nigra and glutamatergic afferents from all parts of the cortex (Gerfen, 1992). Both dopamine (DA) and glutamate play major roles in the pathophysiology of Parkinson's disease (PD) (Calabresi et al., 2007; Chase and Oh, 2000). Loss of nigrostriatal DA fibers causes the main motor features of PD (Morrish et al., 1996) which can be pharmacologically treated with the DA precursor, L-DOPA. The medication remains the most effective treatment for PD but causes motor fluctuations and dyskinesia (abnormal involuntary movements) in up to 80% of the patients after a few years of treatment (Fabbrini et al., 2007). In both patients and animal models, L-DOPA-induced dyskinesia (LID) has been associated with plastic changes in pre-synaptic as well as post-synaptic neuronal targets in the striatum, including abnormal activation of key signaling kinases (Bezard et al., 2005; Picconi et al., 2003; Santini et al., 2007; Westin et al., 2007), elevated extracellular levels of glutamate (Robelet et al., 2004) and DA (Lindgren et al., 2010), and abnormal intracellular trafficking of DA and glutamate receptor subunits (Berthet and Bezard, 2009; Chase et al., 2000; Gardoni et al., 2006; Hallett et al., 2005; Silverdale et al., 2010). All these changes point to dysfunctional interactions between DA and glutamate neurotransmission in LID (Cenci and Lindgren, 2007; Cenci and Lundblad, 2006; Chase et al., 2000). Because antagonism of DA receptors may worsen PD motor symptoms, several glutamate receptor antagonists, especially of the ionotropic type, have been proposed for a pharmacological treatment of LID (Fox et al., 2006). Antagonists of ionotropic receptors may, however, induce cognitive and psychiatric impairments (Meldrum, 1998). Metabotropic glutamate receptors (mGluR) have received growing attention as potential targets for anti-parkinsonian and antidyskinetic treatments (Conn et al., 2005; Marino and Conn, 2006). Ligands to these receptors can normalize excessive glutamate transmission without

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2010.05.001

blocking the physiological actions of glutamate in the brain (Rouse et al., 2000). Of particular interests is metabotropic glutamate receptor 5 (mGluR5), a G-protein coupled receptor that is positively linked to phosphoinositide hydrolysis (group I mGluRs) (Pin and Duvoisin, 1995). This receptor is highly expressed in striatal medium spiny neurons (Testa et al., 1994) where it plays a key role in modulating the responses mediated by NMDA receptors and L-type calcium channels (reviewed in Gubellini et al., 2004). We have previously shown that the selective mGluR5 antagonist, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine (MTEP), can reduce the severity of LID and inhibit the associated molecular and neurochemical changes in rats with 6-hydroxydopamine (6-OHDA) lesions (Dekundy et al., 2006; Mela et al., 2007; Rylander et al., 2009). However, MTEP has no translational potential. We therefore searched for another mGluR5 antagonist, amenable to clinical use, in order to conduct a comprehensive preclinical validation of the mGluR5 target. Fenobam was under development already in the 1970s as non-benzodiazepine anxiolytic compound with potentially good safety profile (Pecknold et al., 1982) but was first identified as a potent, non-competitive mGluR5 antagonist in 2005 (Porter et al., 2005). It shares the same allosteric modulatory site as the prototypical mGluR5 antagonist, 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Gasparini et al., 1999) and, similar to MPEP, it is a candidate compound for the treatment of fragile X syndrome as well as pain (Berry-Kravis et al., 2009; Jacob et al., 2009; Montana et al., 2009). This study provides evidence of anti-dyskinetic and anti-akinetic effects of fenobam in two well-established animal models of PD. With its favourable profile of motor effects and its amenability to clinical use, fenobam can be regarded as a candidate treatment for motor complications in PD.

Materials and methods

Animals

For the rat experiments, female Sprague–Dawley rats (Harlan, Netherlands), weighing 225–250 g at the beginning of the study were used. They were house in a 12 h dark–light cycle and had food and water *ad libitum*. All the procedures were approved by the Malmö-Lund ethical committee on animal research.

Non-human primate models of PD and LID were produced in male rhesus monkeys (*Macaca mulatta*, Xierxin, Beijing, PR of China; mean weight = 5.3 ± 0.8 kg; mean age = 5 ± 1 years) as in (Aubert et al., 2005). Animals were housed in individual primate cages under controlled conditions of humidity, temperature and light. Food and water were available *ad libitum* and animal care 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 in an AAALAC-accredited facility. Procedures were approved by the Institute of Laboratory Animal Science ethical committee.

Experimental design

In both rats and monkeys, fenobam was tested using two treatment regimens:

1. Acute treatment to animals with already established LID. For this experiment we used a group of chronically L-DOPA-treated dyskinetic rats ($n=14$) or macaques ($n=6$), which were sequentially challenged with ascending doses of fenobam or its vehicle in combination with L-DOPA. In rats, fenobam was tested at the doses of 0, 30 or 100 mg/kg and AIMs were rated with two days of wash-out between consecutive doses (Jacob et al., 2009). In macaques, the doses of fenobam were 0, 5, 10 and 20 mg/kg with three days of wash-out between consecutive doses. Amantadine (20 mg/kg per o.s.) was tested on a separate session as a positive anti-dyskinetic drug-control.
2. Chronic *de novo* treatment. For this purpose we applied a cross-over design with 12 + 12 or 17 + 17 days of treatment in rats and monkeys, respectively. Fenobam was administered at the dose producing maximal anti-dyskinetic effect in the acute experiment (30 mg/kg for rat and 10 mg/kg for monkey). Thirty-three rats with 6-OHDA-lesions were randomized to one of the following four treatment groups; (*i*) L-DOPA + vehicle ($n=10$), (*ii*) L-DOPA + fenobam 30 mg/kg ($n=12$), (*iii*) saline + vehicle ($n=5$) and (*iiii*) saline + fenobam 30 mg/kg ($n=6$). Seven MPTP-lesioned monkeys that had been kept without any drug treatment for a year, were allocated to receive L-DOPA + vehicle ($n=3$) or L-DOPA + fenobam 10 mg/kg ($n=4$). Drugs were administered daily during treatment arm 1. After a wash-out period of two-three days, L-DOPA + vehicle and L-DOPA + fenobam groups were swapped and the treatment resumed for another 12 or 17 days (treatment arm 2).

Rat model of PD

A hemiparkinsonian state was produced in the rat by unilateral injections of 6-OHDA in the ascending nigrostriatal DA bundle according to our well-established methods (Cenci and Lundblad, 2007). Rats were anesthetized with a mixture of Fentanyl® and Dormitor® (20:1, Apoteksbolaget AB, Sweden) and mounted on a stereotaxic frame (Kopf Instruments, Tujunga, USA). A total of 7.5 and 6 µg free-base 6-OHDA (6-Hydroxydopamine hydrochloride, Sigma Aldrich, Sweden, dissolved in 0.02% ascorbate-saline) were injected at the following coordinates (in mm, relative to bregma and dural surface): first injection: A = −4.4, L = −1.2, V = −7.8, tooth bar = +2.4 (2,5 µl); second injection: A = −4.0, L = −0.8, V = −8.0, tooth bar = +3.4 (2,0 µl). At the end of the surgery animals were given Temgesic® (0,167 mg/Kg, Apotekesbolaget AB, Sweden) as analgesic treatment. Two weeks post surgery, an amphetamine-induced rotation test (2.5 mg/kg D-amphetamine i.p. for 90 min recording) was applied to select rats with >5 full turns/min ipsilateral to the lesion, corresponding to >90% striatal DA depletion (Carta et al., 2006). Treatment with L-DOPA started 4–8 weeks after the amphetamine-induced rotation test. The striatal DA depletion was further verified by measuring the immunoreactivity for tyrosine hydroxylase (TH) in the striatum (see. S.1).

Non-human primate model of PD

Monkeys were intoxicated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) hydrochloride (0.2 mg/kg i.v. for 15 days) according to our standard method (Bezard et al., 2001b et). Such regimen of intoxication leads to near complete nigrostriatal denervation once a parkinsonian syndrome has fully developed (Guigoni et al., 2005). After inducing a stable, bilateral parkinsonian syndrome (constant disability scores over two consecutive weeks after at least 8 weeks post-MPTP), 6 monkeys were selected for the acute dose–response study and 7 were kept without any treatment to be later used in the chronic *de novo* study. The six animals to be used in the acute dose–response study received daily oral administration of L-DOPA (Modopar®, L-DOPA/carbidopa, ratio 4:1) for 8 weeks at a tailored dose producing full reversal of parkinsonian symptoms (ranging from 15 to 20 mg/kg/day) (Berton et al., 2009; Bezard et al., 2003; Gold et al., 2007; Munoz et al., 2008) and developed stable and moderate–severe LID (dyskinesia severity grade 2–4).

Behavioural analysis in rats

During the experiment rats were treated with 6 mg/kg L-DOPA methyl ester together with the peripheral DOPA-decarboxylase inhibitor, benserazide-HCl (15 mg/kg, both from Sigma-Aldrich, Sweden), which were dissolved in physiological saline and injected (s.c.) at the volume of 1 ml/kg. Fenobam (N-(3-chlorophenyl)-N′-(4,5-dihydro-1-methyl-4-oxo-1H-imidazole-2-yl)urea (Pecknold