Pharmacology, Biochemistry and Behavior 105 (2013) 105–111

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Tremorolytic effects of safinamide in animal models of drug-induced parkinsonian tremor

Samantha Podurgiel [a], Lyndsey E. Collins-Praino [a], Samantha Yohn [a], Patrick A. Randall [a], Arthur Roach [b], Christophe Lobianco [b], John D. Salamone [a,*]

[a] Dept. of Psychology, University of Connecticut, Storrs, CT 06269-1020, USA
[b] Merck Serono, Merck Serono S.A.-Geneva, 9 Chemin des mines, 1202 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 11 November 2012
Received in revised form 16 January 2013
Accepted 19 January 2013
Available online 27 January 2013

Keywords:
Parkinson's disease
MAO
Dopamine
Acetylcholine
Muscarinic
Alzheimer's disease

ABSTRACT

Safinamide is an α-aminoamide derivative that is currently in Phase III clinical trial development as an add-on therapy to levodopa or dopamine agonists for patients with Parkinson's disease. Safinamide is a monoamine oxidase B inhibitor with additional non-dopaminergic actions. The present experiments were performed to evaluate the ability of safinamide to attenuate parkinsonian motor impairments using the tremulous jaw movement model, an animal model of parkinsonian tremor. In rats, tremulous jaw movements can be induced with dopamine (DA) antagonists, DA depletion, and cholinomimetics, and can be reversed by various antiparkinsonian drugs, including L-DOPA, DA agonists, anticholinergics and adenosine A_{2A} antagonists. In these present experiments, tremulous jaw movements were induced with the anticholinesterase galantamine (3.0 mg/kg IP), the muscarinic agonist pilocarpine (0.5 mg/kg IP), and the dopamine D2 antagonist pimozide (1.0 mg/kg IP). Safinamide significantly reduced the number of tremulous jaw movements induced by galantamine, pilocarpine, and pimozide, with consistent effects across all three drugs at a dose range of 5.0–10.0 mg/kg. The results of this study support the use of safinamide as a treatment for parkinsonian tremor.

1. Introduction

In idiopathic Parkinson's disease, neurodegenerative processes that deplete striatal dopamine (DA) result in the development of motor symptoms including akinesia, bradykinesia, rigidity and tremor (Bernheimer et al., 1973). In addition, several classes of drugs are known to induce parkinsonian symptoms in humans. Administration of antipsychotic drugs that block DA receptors (*e.g.*, haloperidol, chlorpromazine, and pimozide) or deplete striatal DA (*e.g.* reserpine or tetrabenazine) has been shown to induce parkinsonian motor symptoms (Marsden et al., 1975; McEvoy, 1983; Arbaizar et al., 2008). Furthermore, cholinomimetic drugs are known to be tremorogenic (Brimblecombe, 1975; Dronfield et al., 2000; Liston et al., 2004; Salamone et al., 2001), and several clinical studies have reported that cholinomimetics can induce or exacerbate parkinsonian symptoms, including tremor, in humans (Aarsland et al., 2003; Arai, 2000; Bourke and Drukenbrod, 1998; Cabeza-Alvarez et al., 1999; Duvoisin, 1967; Gurevich et al., 2006; Iwasaki et al., 1988; Kao et al., 1993; Keltner, 1994; McSwain and Forman, 1995; Ott and Lannon, 1992; Shea et al., 1998; Song et al., 2008). The most common treatment for idiopathic Parkinson's disease is the DA precursor L-DOPA, but there are complications associated with the long-term use of L-DOPA (*e.g.*, on–off effects and dyskinesias; Salamone, 2010b), and parkinsonian symptoms also are treated by a number of other dopaminergic and non-dopaminergic agents, including DA agonists, muscarinic acetylcholine antagonists (Aquilonius, 1980; McEvoy, 1983), and amantadine. Novel therapeutic approaches include adenosine A_{2A} antagonists, gene therapies, and various surgical procedures intended to restore neurochemical balance in the basal ganglia circuitry (Salamone, 2010a; Hauber et al., 2001).

Monoamine oxidase B (MAO-B) inhibitors have also been employed as a treatment for parkinsonism (Moussa et al., 2006; Onofrj et al., 2008; Schapira, 2010). MAO-B is one of the key enzymes responsible for dopamine metabolism in the brain (Moussa et al., 2006). Selegiline and rasagiline are potent, irreversible MAO-B selective inhibitors that are presently available for the treatment of PD (Onofrj et al., 2008; Schapira, 2010). Safinamide, ((S)-(+)-2-[4-(3-fluorobenzyloxybenzylamino)pro-panamide]methanesulfonate (1:1 salt), is a water soluble, α-aminoamide derivative with multiple actions (Marzo et al., 2004; Fariello, 2007; Onofrj et al., 2008; Schapira, 2010; Stocchi et al., 2012). Safinamide is a potent and reversible inhibitor of MAO-B across multiple preparations (*e.g.* human platelets and rat brain mitochondria), with greater selectivity for MAO-B over MAO-A than selegiline and rasagiline (Marzo et al., 2004; Caccia et al., 2006; Fariello, 2007; Onofrj et al., 2008; Schapira, 2010; Stocchi et al., 2012). Additionally, safinamide reduces dopamine uptake, blocks voltage-dependent sodium channels, modulates N-type calcium channels, and reduces glutamate release (Marzo et al., 2004; Fariello, 2007; Onofrj et al., 2008; Schapira, 2010; Stocchi et al., 2012). By doing so, animal studies have revealed that safinamide exerts neuroprotectant,

* Corresponding author at: Division of Behavioral Neuroscience, Dept. of Psychology, University of Connecticut, Storrs, CT 06269-1020, USA.

0091-3057/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pbb.2013.01.015

anticonvulsant, and antiparkinsonian properties (Salvati et al., 1999; Fariello et al., 2000; Caccia et al., 2006; Onofrj et al., 2008; Gregoire et al., 2010). Safinamide has an excellent therapeutic and safety margin as assessed in studies with normal human volunteers and patients, and is currently in Phase III clinical trial development as an add-on therapy to levodopa or dopamine agonists for early PD patients (Marzo et al., 2004; Onofrj et al., 2008; Schapira, 2010; Stocchi et al., 2004, 2012; Schapira et al., 2013).

Several animal models have been used to assess various motor functions related to parkinsonism (Avila et al., 2009; Castañeda et al., 2005; Pollack and Thomas, 2010). Although resting tremor is one of the cardinal symptoms of parkinsonism, relatively little information is known about the neural mechanisms underlying tremorogenesis or its treatment (Bergman and Deuschl, 2002; Deuschl et al., 2001; Fishman, 2008; Binder et al., 2009), and research employing animal models of tremor also can contribute greatly to our understanding of the neurochemical regulation of tremorogenesis (Miwa, 2007; Salamone et al., 1998; Wilms et al., 1999). For this reason, the present studies used the tremulous jaw movement model, which is a rodent model of parkinsonian tremor that has been extensively employed (Cenci et al., 2002; Cousins et al., 1998; Ishiwari et al., 2005; Miwa et al., 2008, 2009; Rodriguez Diaz et al., 2001; Salamone et al., 1990, 1998, 2001, 2005, 2008a, 2008b; Simola et al., 2004, 2006; Vanover et al., 2008). These movements are defined as repetitive vertical deflections of the lower jaw that resemble chewing but are not directed at a particular stimulus (Salamone et al., 1998). As shown by studies using videotape analyses or electromyographic methods, these movements occur largely within the 3–7 Hz frequency range that is characteristic of parkinsonian resting tremor (Cousins et al., 1998; Finn et al., 1997; Ishiwari et al., 2005; Mayorga et al., 1997), and can be induced by a number of conditions that parallel the neurochemistry of the pathology of parkinsonism, including striatal DA depletion, DA antagonism, anticholinesterases and muscarinic agonists (Baskin and Salamone, 1993; Betz et al., 2005; Cousins et al., 1998; Finn et al., 1997; Ishiwari et al., 2005; Jicha and Salamone, 1991; Mayorga et al., 1997; Rodriguez Diaz et al., 2001; Salamone and Baskin, 1996; Salamone et al., 1990, 1998, 2005, 2008a; Steinpreis et al., 1993; Trevitt et al., 1998). Dopaminergic antiparkinsonian drugs such as apomorphine, L-DOPA, bromocriptine, pergolide, and ropinirole can reduce cholinomimetic-induced tremulous jaw movements (Cousins et al., 1997; Salamone et al., 2005), and their potency for suppressing cholinomimetic-induced tremulous jaw movements is highly correlated ($r = 0.88$) with the clinical potency of these drugs for reducing parkinsonian tremor in humans (Salamone et al., 2005). Tremulous jaw movements are sensitive to several other classes of antiparkinsonian drugs, including muscarinic antagonists and adenosine A_{2A} antagonists (Baskin and Salamone, 1993; Betz et al., 2007, 2009; Correa et al., 2004; Cousins et al., 1997; Salamone et al., 1998, 2008a; Simola et al., 2004, 2006; Steinpreis et al., 1993; Tronci et al., 2007).

The present experiments examined the ability of safinamide to attenuate drug-induced tremulous jaw movements in rats. Because cholinomimetics are well known tremorogenic agents (Brimblecombe, 1975; Salamone et al., 2001; Collins-Praino et al., 2011), the first two experiments will employ cholinomimetics that are known to induce tremulous jaw movements in order to assess the effects of safinamide. The first experiment assessed the ability of safinamide to reverse tremulous jaw movements induced by the anticholinesterase galantamine, which is a second generation anticholinesterase with improved clinical profile in Alzheimer's disease patients, but which also can produce Parkinsonian side effects including tremor (Collins et al., 2011). The second experiment examined the ability of safinamide to attenuate tremulous jaw movements induced by the muscarinic agonist pilocarpine (Collins et al., 2010a). The final experiment studied the ability of safinamide to reverse the effects of the DA D2 antagonist pimozide on tremulous jaw movements and locomotor suppression, under the same conditions used previously for the assessment of other antiparkinsonian agents (Salamone et al., 2008a). These conditions (*i.e.*, repeated administration of 1.0 mg/kg pimozide) were optimized for induction of tremulous jaw movements (Ishiwari et al., 2005; Salamone et al., 2008a), but also allow for assessment of locomotion. It was hypothesized that safinamide would be able to reverse the tremorogenic effects of galantamine, pilocarpine, and pimozide.

2. Materials and methods

2.1. Animals

A total of 118 adult male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with no prior drug experience were used in the present experiments. The rats weighed 350–450 g during the course of the experiment and had *ad libitum* access to lab chow and water. They were group-housed in a colony that was maintained at approximately 23 °C and had a 12-hour light/dark cycle (lights on at 0700 h). These studies were conducted according to University of Connecticut and NIH guidelines for animal care and use.

2.2. Drug treatment procedures and dose selection

Galantamine hydrobromide was obtained from Tocris Bioscience (Bristol, UK) and dissolved in 0.9% saline. Pilocarpine and pimozide were purchased from Sigma Aldrich Chemical (St. Louis, MO). Pilocarpine was dissolved in 0.9% saline, and pimozide was dissolved in a 0.3% tartaric acid solution (final pH = 4.0). Safinamide, (S)-(+)-2-[4-(3-fluorobenzyloxybenzylamino)pro-panamide]methanesulfonate (1:1 salt) is a water soluble, α-aminoamide derivative. Safinamide was obtained from Merck Serono International S.A. (Geneva, Switzerland) and was dissolved in 0.9% saline, which was also used as the vehicle control. An acute dose of 3.0 mg/kg (IP) galantamine or 0.5 mg/kg (IP) pilocarpine was used for the studies examining galantamine or pilocarpine-induced tremulous jaw movements. The selection of these doses was based on previously published experiments showing induction of jaw movements at these doses (Collins et al., 2010a; Collins-Praino et al., 2011). Subchronic 1.0 mg/kg (IP) pimozide treatment was used for the study examining tremulous jaw movements and locomotion. This treatment procedure was based upon previously published experiments showing induction of jaw movements at this dose (Ishiwari et al., 2005; Betz et al., 2007, 2009; Salamone et al., 2008a; Collins et al., 2010b). Subchronic 1.0 mg/kg (IP) pimozide treatment is optimized for the production of jaw movement activity and also allows for the parallel assessment of locomotion. The procedure of screening animals by assessing them for tremulous jaw movements the day before the drug challenge day was the same as that used in previous studies (Ishiwari et al., 2005; Salamone et al., 2008a; Collins et al., 2010b). This was done in order to ensure a robust jaw movement response on the drug challenge day. None of the animals were rejected because of a poor jaw movement response to pimozide on day 7 (*i.e.*, less than 15 movements in 5 min). The doses of safinamide chosen were based upon extensive pilot work. A vehicle/vehicle control condition was used in experiment 3, but not in experiments 1 and 2, because cholinomimetics induce a much higher level of jaw movement activity than pimozide, and also because a vehicle control was needed for the effect of pimozide in the locomotion experiment.

2.3. Behavioral procedures

2.3.1. Tremulous jaw movements

Observations of rats took place in a 30 × 30 × 30 cm clear Plexiglas chamber with a wire mesh floor, which was elevated 42 cm from the table top. This allowed for the viewing of the animal from several angles, including underneath. Tremulous jaw movements were defined as rapid vertical deflections of the lower jaw that resembled chewing but were not directed at any particular stimulus (Salamone et al.,