Biochemical Pharmacology 86 (2013) 1153–1162

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

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

Multiple CNS nicotinic receptors mediate L-dopa-induced dyskinesias: Studies with parkinsonian nicotinic receptor knockout mice☆

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ARTICLE INFO

Article history:
Received 26 April 2013
Accepted 25 June 2013
Available online 4 July 2013

Keywords:
Dyskinesias
Nicotine
Nicotinic receptors
Parkinson's disease
Striatum

ABSTRACT

Accumulating evidence supports the idea that drugs acting at nicotinic acetylcholine receptors (nAChRs) may be beneficial for Parkinson's disease, a neurodegenerative movement disorder characterized by a loss of nigrostriatal dopaminergic neurons. Nicotine administration to parkinsonian animals protects against nigrostriatal damage. In addition, nicotine and nAChR drugs improve L-dopa-induced dyskinesias, a debilitating side effect of L-dopa therapy which remains the gold-standard treatment for Parkinson's disease. Nicotine exerts its antidyskinetic effect by interacting with multiple nAChRs. One approach to identify the subtypes specifically involved in L-dopa-induced dyskinesias is through the use of nAChR subunit null mutant mice. Previous work with β2 and α6 nAChR knockout mice has shown that α6β2* nAChRs were necessary for the development/maintenance of L-dopa-induced abnormal involuntary movements (AIMs). The present results in parkinsonian α4 nAChR knockout mice indicate that α4β2* nAChRs also play an essential role since nicotine did not reduce L-dopa-induced AIMs in such mice. Combined analyses of the data from α4 and α6 knockout mice suggest that the α6α4β2β3 subtype may be critical. In contrast to the studies with α4 and α6 knockout mice, nicotine treatment did reduce L-dopa-induced AIMs in parkinsonian α7 nAChR knockout mice. However, α7 nAChR subunit deletion alone increased baseline AIMs, suggesting that α7 receptors exert an inhibitory influence on L-dopa-induced AIMs. In conclusion, α6β2*, α4β2* and α7 nAChRs all modulate L-dopa-induced AIMs, although their mode of regulation varies. Thus drugs targeting one or multiple nAChRs may be optimal for reducing L-dopa-induced dyskinesias in Parkinson's disease.

1. Introduction

Parkinson's disease is a progressive neurodegenerative movement disorder characterized by rigidity, tremor and bradykinesia [1–8]. These symptoms arise because of a loss of nigrostriatal dopaminergic neurons. Dopamine precursor therapy with L-dopa alleviates many of the parkinsonian motor symptoms; however, abnormal involuntary movements (AIMs) or dyskinesias arise in most patients with extended treatment. These dyskinesias can range from mild to so severe that they interfere with routine functions of daily living. Presently there are few treatment options for L-dopa-induced dyskinesias. Dopamine agonists are used in early Parkinson's disease as these drugs produce fewer dyskinesias, but they are less effective than L-dopa with disease progression. Amantadine is the only clinically approved pharmacological agent for the treatment of L-dopa-induced dyskinesias; however, its effects are limited and diminish with time [9–12]. Deep brain stimulation has proved very successful for some Parkinson's disease patients; however, this is a serious intervention with all the potential adverse effects associated with brain surgery [13,14]. Extensive research is therefore underway to identify drugs to reduce dyskinesias by targeting CNS neurotransmitter systems directly or indirectly associated with striatal function [1–8]. This includes the nicotinic acetylcholine receptor (nAChR) system which is anatomically and functionally linked to the dopaminergic system [15].

NAChRs, which mediate the action of acetylcholine, are widely expressed in the striatum as well as other brain regions linked to motor function [15]. These ligand-gated ion channels are composed of different combinations of α and β subunits, with the most common subtypes in striatum being α4β2*, α6β2* and α7 nAChRs. The asterisk indicates the possible presence of other subunits in the receptor complex. The populations most

☆ This work was supported by NIH grants NS59910, NS65851, and DA015663.
Abbreviations: α-CtxMII, α-conotoxinMII; nAChR, nicotinic acetylcholine receptor; *, the possible presence of other subunits in the receptor complex.
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0006-2952/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2013.06.027

prominently expressed on dopaminergic terminals in the striatum, a primary area that degenerates in Parkinson's disease, are the α4β2* and α6β2* nAChRs [15–17]. In addition, α4β2* nAChRs are located on striatal GABAergic neurons [18]. Although expressed at a lower density, α7 nAChRs are also present in the striatum on cortical glutamatergic afferents [19]. Thus there is a rationale for considering that all subtypes may influence functions linked to the nigrostriatal system, such as the development of L-dopa-induced dyskinesias.

Consistent with this idea, previous studies have shown that nicotine reduces L-dopa-induced AIMs in parkinsonian rats, mice and monkeys by 50–70% [20–23]. Nicotine treatment attenuated AIMs when administered by injection, drinking water or mini-pump, with the latter two readily amenable to use in patients either orally or via a patch. Several lines of evidence indicate that nicotine reduces L-dopa-induced dyskinesias by acting at nAChRs. Varenicline, an agonist that interacts with multiple nAChRs [24–30], decreased L-dopa-induced AIMs in 6-hydroxydopamine (6-OHDA)-lesioned rats by ~50%. The nAChR antagonist mecamylamine ameliorated L-dopa-induced AIMs to a similar extent as nicotine. However, its effects on AIMs were not additive with those of nicotine, further suggesting that nicotine acts at nAChRs [22]. Moreover, 5-iodo-A-85380 (A-85380), an agonist that acts selectively at α4β2* and α6β2* nAChRs, reduced AIMs supporting the idea that the β2* nAChR subtype is important in modulating L-dopa-induced AIMs [31–33].

Further evidence for a role of β2* nAChRs stems from experiments with nAChR null mutant mice, which have thus far focused on β2 and α6 nAChR subunit knockout animals. L-dopa-induced AIMs were lower in β2 nAChR subunit knockout mice with a 6-OHDA lesion compared to wild type [34]. Moreover, nicotine treatment did not reduce AIMs in the knockout mice, indicating that β2* nAChRs were essential [34]. Similar results were observed with α6 nAChR knockout mice suggesting that α6β2* nAChRs are involved in the antidyskinetic effect of nicotine [35].

The present studies were done to investigate whether α4β2* and α7 nAChRs also play a role in L-dopa-induced AIMs. To approach this, we used α4 and α7 nAChR null mutant mice. The results show that α4β2* and α7 nAChRs also regulate AIMs, although in a somewhat different fashion from α6β2* nAChRs and from one another. Thus, drugs targeting α6β2*, α4β2* and/or α7 nAChRs have the potential to influence the occurrence of L-dopa-induced dyskinesias.

2. Materials and methods

2.1. Animals

Mice with nAChR subunit null mutations (α4, originally obtained from Dr. John Drago, University of Melbourne, Australia; α7, originally obtained from Dr. Arthur Beaudet, Baylor College of Medicine, Houston, USA) were bred onto the C57Bl/6 background for a minimum of 10 generations. They were maintained and produced by heterozygous matings at the Institute for Behavioral Genetics, University of Colorado, Boulder. All care and genotyping procedures were in accordance with the NIH Guide and approved by the Animal Care and Use Committee of the University of Colorado, Boulder. Mice were weaned at 21 days of age and housed 1–5 per cage with same-sex littermates, under a 12 h light/dark cycle with free access to food and water. The mice were genotyped by PCR as previously described [36].

Five wk or more after weaning, the mice were couriered to SRI International, where they were housed 1–5 per cage with same-sex littermates in a temperature- and humidity-controlled environment under a 12 h light/dark cycle with free access to food and water. After an initial acclimation period (2–3 d), the mice were anesthetized with isofluorane (3%) and placed in a Kopf stereotaxic instrument. A burr hole was then drilled through the right side of the skull at the following coordinates relative to Bregma and the dural surface: anteroposterior, −1.2; lateral, −1.2; ventral, 4.75. The mice next received a unilateral intracranial injection of 3 μg 6-OHDA (Sigma–Aldrich, St. Louis, MO, USA) (in 1 μl) into the medial forebrain bundle to lesion the dopaminergic nigrostriatal pathway, as previously described [35]. Our previous studies show that such a lesion results in ~90% reduction in the striatal dopamine transporter [35]. All procedures were approved by the Institutional Animal Care and Use Committee at SRI International in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Drug treatment

Nicotine (Sigma–Aldrich, St. Louis, MO, USA) was administered via the drinking solution containing 2% saccharin (Sigma Aldrich, St. Louis, MO, USA). The mice were first given only saccharin. Two days later nicotine (free base, 25 μg/ml) was added for 2 days, with the dose increased to 50 μg/ml on day 3, to 100 μg/ml on day 5, to 200 μg/ml on day 8, to a final dose on day 10 of 300 μg/ml (nicotine titration phase), as previously described [34,35,37–39].

L-Dopa methyl ester (L-dopa) and benserazide hydrochloride were both purchased from Sigma–Aldrich (St. Louis, MO, USA). L-Dopa (3 mg/kg) and benserazide (15 mg/kg) were dissolved in saline and injected sc once daily throughout the study, as previously described [34,35,40]. Benserazide is an aromatic amino acid decarboxylase inhibitor given to inhibit the breakdown of L-dopa in the periphery.

2.3. Behavioral testing

L-Dopa-induced AIMs were rated as described previously [34,35,40]. Individual mice were placed in a cylinder and rated every 15 min for 1 min over a 2 h period. Each AIM subtype was scored on a frequency scale from 0 to 4 (0 = no dyskinesia; 1 = occasional dyskinesia displayed for <50% of the observation time; 2 = sustained dyskinesia displayed for >50% of the observation time; 3 = continuous dyskinesia; 4 = continuous dyskinesia not interruptible by external stimuli). The animals were also rated on an amplitude scale, consisting of two levels. Level A indicated oral AIMs without tongue protrusion, forelimb AIMs without the shoulder engaged or axial AIMs with body twisting <60°. Level B indicated oral AIMs with tongue protrusion, forelimb AIMs with the shoulder engaged or axial AIMs with body twisting > 60°. The integrated scores for frequency and amplitude of AIMs used for data analysis were calculated as 1A = 1, 1B = 2, 2A = 2, 2B = 4, 3A = 4, 3B = 6, 4A = 6, 4B = 8. This allowed for scores for any one component (oral, forelimb or axial) to range from 0 to 8, with a maximum possible total score per time point of 24. All rating was done in a blinded fashion.

To evaluate parkinsonism we used the forelimb asymmetry test [34,35,41,42]. Forelimb use was assessed in a plexiglass cage for a 3 min period, with mirrors strategically placed to observe paw movement. Wall exploration was expressed in terms of the % use of the impaired forelimb (contralateral) compared to the total number of limb use movements. All rating was done in a blinded fashion.

2.4. Plasma cotinine levels

Blood (~0.2 ml) was taken from the ocular vein under isofluorane anesthesia and plasma prepared. Cotinine levels were determined throughout the study using an EIA kit (Orasure Technologies, Bethlehem, PA).