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EVIDENCE FOR A ROLE FOR α6* nAChRs IN L-DOPA-INDUCED DYSKINESIAS USING PARKINSONIAN α6* nAChR GAIN-OF-FUNCTION MICE

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Abstract—L-Dopa-induced dyskinesias (LIDs) are a serious side effect of dopamine replacement therapy for Parkinson's disease. The mechanisms that underlie LIDs are currently unclear. However, preclinical studies indicate that nicotinic acetylcholine receptors (nAChRs) play a role, suggesting that drugs targeting these receptors may be of therapeutic benefit. To further understand the involvement of α6β2* nAChRs in LIDs, we used gain-of-function α6* nAChR (α6L9S) mice that exhibit a 20-fold enhanced sensitivity to nAChR agonists. Wildtype (WT) and α6L9S mice were lesioned by unilateral injection of 6-hydroxydopamine (6-OHDA, 3 μg/ml) into the medial forebrain bundle. Three to 4 wk later, they were administered L-dopa (3 mg/kg) plus benserazide (15 mg/kg) until stably dyskinetic. L-dopa-induced abnormal involuntary movements (AIMs) were similar in α6L9S and WT mice. WT mice were then given nicotine in the drinking water in gradually increasing doses to a final 300 μg/ml, which resulted in a 40% decline AIMs. By contrast, there was no decrease in AIMs in α6L9S mice at a maximally tolerated nicotine dose of 20 μg/ml. However, the nAChR antagonist mecamylamine (1 mg/kg ip 30 min before L-dopa) reduced L-dopa-induced AIMs in both α6L9S and WT mice. Thus, both a nAChR agonist and antagonist decreased AIMs in WT mice, but only the antagonist was effective in α6L9S mice. Since nicotine appears to reduce LIDs via desensitization, hypersensitive α6β2* nAChRs may desensitize less readily. The present data show that α6β2* nAChRs are key regulators of LIDs, and may be useful therapeutic targets for their management in Parkinson's disease.

Key words: dyskinesia, L-dopa, nicotine, 6-hydroxydopamine, Parkinson's disease.

INTRODUCTION

Long-term L-dopa use is complicated by the emergence of abnormal involuntary movements (AIMs) or dyskinesias, for which there are currently few treatments (Huot et al., 2011; Connolly and Lang, 2014). There is thus a critical unmet need for therapies to reduce L-dopa-induced dyskinesias (LIDs). Preclinical studies suggest a compelling role for the nicotinic cholinergic system (Quik et al., 2014). Nicotine administration alleviated LIDs up to 60% in a variety of parkinsonian animal models, suggesting it may represent a useful treatment option (Quik et al., 2007; Bordia et al., 2008; Huang et al., 2011a).

Nicotine generally exerts its effects in the brain by acting at nicotinic acetylcholine receptors (nAChRs), of which there are several subtypes. The primary subtypes in the striatum, a region prominently affected in Parkinson's disease and linked to LIDs, are the α4β2*, α6β2* and α7 nAChRs. The asterisk indicates the possible presence of other subunits in the receptor complex (Millar and Gotti, 2009; Quik and Wonnacott, 2011). Two approaches have proved useful in delineating the nAChRs that mediate the nicotine-induced decline in LIDs. One of these involves the use of drugs targeting select nAChRs. Work with α7 nAChR agonists showed that administration of ABT-107 or AQW051 to monkeys led to ~60% decline in LIDs (Di Paolo et al., 2014; Zhang et al., 2014b). β2* nAChR agonists, which act at both α4β2* and α6β2* subtypes, also significantly reduced LIDs in parkinsonian rats and monkeys. Varenicline, ABT-089, ABT-894, TC-8831, as well as other TC-agonists, attenuated LIDs by 30–60% (Huang et al., 2011b; Johnston et al., 2013; Quik et al., 2013a; Zhang et al., 2013, 2014a). Interestingly, the general nAChR antagonist mecamylamine also reduced LIDs to a similar extent as nicotine and nAChR agonists (Bordia et al., 2010). This latter finding led to the suggestion that agonists may reduce LIDs by a nAChR desensitization block, a mechanism through which agonists also

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Abbreviations: α-CtxMII, α-conotoxinMII; *, denotes the possible presence of other subunits in the receptor complex; ^{125}I-RTI-121, ^{125}I-3β-(4-iodophenyl)tropane-2β-carboxylic acid isopropyl ester; 6-OHDA, 6-hydroxydopamine; AIMs, abnormal involuntary movements; BSA, bovine serum albumin; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LIDs, L-dopa-induced dyskinesias; NAChRs, nicotinic acetylcholine receptors; WT, wildtype.

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modulate other behaviors (Picciotto et al., 2008; Buccafusco et al., 2009). The idea that LIDs are reduced because of a nAChR blockade is also consistent with a recent study which showed that ablation of striatal cholinergic interneurons, which results in a loss of acetylcholine, markedly reduced LIDs (Won et al., 2014).

Studies with genetically modified mice lend further support to the idea that multiple nAChRs are involved in the regulation of LIDs. Deletion of the α7 nAChR led to an increase in baseline LIDs, although it did not affect the antidyskinetic effect of nicotine (Quik et al., 2013b). By contrast, mice lacking β2* nAChRs, that is, both the α4β2* and α6β2* subtypes, exhibited a 50% decline in baseline LIDs. In addition, nicotine treatment no longer reduced LIDs in β2 null mutant mice. Selective subunit deletion of only the α4 nAChR subunit resulted in a loss of the antidyskinetic effect of nicotine with no change in baseline LIDs. By contrast, deletion of only the α6 nAChR subunit led to a decline in baseline LIDs together with a loss of the antidyskinetic effect of nicotine. These latter findings suggest an important role for α6β2* nAChRs in LIDs (Quik et al., 2012).

The objective of the current study was to use gain-of-function α6L9S mice to further explore the role of α6β2* nAChRs in LIDs. These mice express an α6* nAChR subunit in which the Leu 9′ residue in the M2 transmembrane domain is mutated to a Ser (Drenan et al., 2008). This mutation results in an α6β2* nAChR channel hypersensitive to endogenous acetylcholine or nAChR agonists, with a consequent increase in dopaminergic function (Drenan et al., 2008, 2010; Wang et al., 2014). In addition, transgenic mice expressing α6L9S nAChRs exhibit a variety of enhanced ambulatory behaviors, including walking, turning and rearing (Drenan et al., 2010). The present data using such transgenic mice further support a role for α6β2* nAChRs in LIDs.

EXPERIMENTAL PROCEDURES

Animals and nigrostriatal lesioning

Gain-of-function α6L9S mice and their wildtype (WT) littermates were bred, raised and genotyped at Purdue University, as described (Drenan et al., 2008). Adult male mice (20–35 g) were then shipped to SRI for lesioning, behavioral and biochemical studies. Upon arrival, mice were group housed in a room with controlled temperature and humidity, and a 12-h light/dark cycle. The mice had free access to food and water. After 1 wk of acclimation, the mice were lesioned by unilateral intracranial injection of 6-hydroxydopamine (6-OHDA) (Sigma–Aldrich Co., St. Louis, MO, USA) into the right medial forebrain bundle, as described (Lundblad et al., 2004, 2005; Huang et al., 2011a; Quik et al., 2012, 2013b). 6-OHDA (3 μg free base/μl in 0.9% saline containing 0.02% ascorbic acid) was stereotaxically injected under isofluorane anesthesia at the following site: anteroposterior, −1.2; lateral, −1.2; ventral, 4.75, relative to the bregma. The cannula was slowly lowered into the brain, with 6-OHDA delivered over a 2-min period. The cannula was maintained at the target site for an additional 2 min, followed by a 2-min removal period. Buprenorphine (0.3 mg/kg) was injected subcutaneously for post-operative pain management and a 0.5-ml aliquot of physiological saline to minimize dehydration. Following surgery, a 20% sucrose solution containing ground food pellets was placed at the bottom of the cage to assist feeding for 1–2 wk, as necessary.

All procedures were approved by the Institutional Animal Care and Use Committee in accordance with the NIH. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Behavioral measurements

Three weeks after 6-OHDA lesioning, mice were assessed for nigrostriatal damage using the forelimb use asymmetry test (cylinder test) (Fig. 1). Mice were placed singly in a transparent cylinder and rated for 3 min for exploratory activity by a blinded rater (Huang et al., 2011a; Quik et al., 2012, 2013b). Contacts with the container wall using the impaired forelimb (contralateral to the lesion) were expressed as % of total forelimb contacts.

Mice were then administered L-dopa (3 mg/kg) plus benserazide (15 mg/kg) (both from Sigma–Aldrich Co., St. Louis, MO, USA) subcutaneously once daily 3 d per wk (Fig. 1), as described (Huang et al., 2011a; Quik et al., 2012, 2013b). Two weeks later, they were assessed for L-dopa-induced AIMs. Briefly, mice were injected with L-dopa and placed in separate clear containers. Ten minutes after the injection they were scored individually for 1 min every 15 min over a 2-h period by a blinded rater. Each AIM subtype (oral, forelimb, and axial) was scored on a frequency scale ranging from 0 to 4 (0 = no AIMs; 1 = occasional AIMs displayed <50% of the observation time; 2 = sustained AIMs for >50% of the observation time; 3 = continuous AIMs; 4 = continuous AIMs not interruptible by external stimuli). Each of the AIM subtypes was also scored for amplitude designated as A or B, with "A" representing oral AIMs without tongue protrusion, forelimb AIMs without shoulder involvement, and axial AIMs with body twisting <60°. "B" represented oral AIMs with tongue protrusion, forelimb AIMs with shoulder involvement or axial AIMs with body twisting >60°. The total score per mouse at any time point was calculated as follows; 1A = 1, 1B = 2, 2A = 2, 2B = 4, 3A = 4, 3B = 6, 4A = 6, 4B = 8, with a score for any one component (axial, oral or forelimb) ranging from 0 to 8. Therefore, the maximum possible score for each mouse was 192 (max score per session = 24, with eight sessions over the 2-h period).

Drug treatments

After 3 wk of L-dopa treatment when dyskinesias are stably expressed, α6L9S and WT mice were acclimated to 2% saccharin drinking solution for 2 d. Saccharin was necessary to mask the bitter of taste of nicotine (Fig. 1). The two genotypes were then divided into two groups each, with one receiving drinking water with only saccharin and the other saccharin-containing nicotine. The mean total dyskinesia scores were similar in all groups. For the WT mice, nicotine treatment was started at a dose of 25 μg/ml for 2 d, 50 μg/ml for 2 d, 100 μg/ml for 3 d, 200 μg/ml for 3 d and then 300 μg/ml, at