European Journal of Pharmacology 701 (2013) 1–6

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Beta2-containing nicotinic acetylcholine receptors mediate calcium/calmodulin-dependent protein kinase-II and synapsin I protein levels in the nucleus accumbens after nicotine withdrawal in mice

Kia J. Jackson [a], M. Imad Damaj [b,*]

[a] Virginia Institute for Psychiatric and Behavioral Genetics, Department of Psychiatry, Virginia Commonwealth University, 800 E. Leigh St., Suite 390A, Richmond, VA 23219, USA
[b] Department of Pharmacology/Toxicology, Virginia Commonwealth University, 410N, 12th St., Box 980613, Richmond, VA 23219, USA

ARTICLE INFO

Article history:
Received 14 September 2012
Received in revised form
6 December 2012
Accepted 7 December 2012
Available online 10 January 2013

Keywords:
CaMKII
Calcium signaling
Nicotine withdrawal
Nucleus accumbens
Nicotine dependence
Synapsin I
Nicotinic receptors

ABSTRACT

Nicotinic acetylcholine receptors are calcium-permeable and the initial targets for nicotine. Studies suggest that calcium-dependent mechanisms mediate some behavioral responses to nicotine; however, the post-receptor calcium-dependent mechanisms associated with chronic nicotine and nicotine withdrawal remain unclear. The proteins calcium/calmodulin-dependent protein kinase II (CaMKII) and synapsin I are essential for neurotransmitter release and were shown to be involved in drug dependence. In the current study, using pharmacological techniques, we sought to (a) complement previously published behavioral findings from our lab indicating a role for calcium-dependent signaling in nicotine dependence and (b) expand on previously published acute biochemical and pharmacological findings indicating the relevance of calcium-dependent mechanisms in acute nicotine responses by evaluating the function of CaMKII and synapsin I after chronic nicotine and withdrawal in the nucleus accumbens, a brain region implicated in drug dependence. Male mice were chronically infused with nicotine for 14 days, and treated with the β2-selective antagonist dihydro-β-erythroidine (DHβE), or the α7 antagonist, methyllycaconitine citrate (MLA) 20 min prior to dissection of the nucleus accumbens. Results show that phosphorylated and total CaMKII and synapsin I protein levels were significantly increased in the nucleus accumbens after chronic nicotine infusion, and reduced after treatment with DHβE, but not MLA. A spontaneous nicotine withdrawal assessment also revealed significant reductions in phosphorylated CaMKII and synapsin I levels 24 h after cessation of nicotine treatment. Our findings suggest that post-receptor calcium-dependent mechanisms associated with nicotine withdrawal are mediated through β2-containing nicotinic receptors.

1. Introduction

Nicotine-associated behaviors are mediated through nicotinic acetylcholine receptors, which are calcium-permeable, and the initial targets for nicotine. Upon nicotine binding, a direct influx of calcium through nicotinic receptors leads to an indirect calcium influx through voltage-gated calcium channels and intracellular calcium stores (Rathouz and Berg, 1994; Dajas-Bailador et al., 2002). The subsequent rise in intracellular calcium induces activation of various downstream second-messengers, including calcium/calmodulin-dependent protein kinase II (CaMKII), one of the most abundant proteins in neurons (Deisseroth et al., 1998), and a protein involved in several essential processes, including induction of long term potentiation (Lisman et al., 2002) and neurotransmitter release (Schulman and Hanson, 1993). CaMKII, in turn, activates various substrates, including synapsin I, a presynaptic vesicle-associated protein essential for neurotransmitter release and phosphorylated by CaMKII at Ser-566 and Ser-603 (De Camilli et al., 1990; Hilfiker et al., 1999).

Activation of these post-receptor calcium-dependent signaling cascades is involved in nicotine-mediated responses. Acute nicotine-induced increases in CaMKII activity are mediated through β2-containing nicotinic receptors in brain regions implicated in drug dependence, including the ventral tegmental area, nucleus accumbens, and amygdala (Jackson et al., 2009d). An acute systemic injection of nicotine also elevates CaMKII in the spinal cord (Damaj, 2000, 2007). Further, L-type calcium channel blockers and CaMKII inhibitors block development and expression of nicotine-induced antinociception at the spinal level (Damaj, 2005). Calcium-dependent mechanisms are also involved in physical and affective nicotine withdrawal behaviors (Biala and Weglinska, 2005; Jackson and Damaj, 2009a). In addition to CaMKII, synapsin I activity is also increased in the nucleus

* Correspondence to: 1220 East Broad Street, Molecular Medicine Research Building Room 3044, P.O. Box 980613, Richmond, Virginia 23298-0613, USA. Tel.: +1 804 828 1676.
E-mail address: mdamaj@vcu.edu (M. Imad Damaj).

0014-2999/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2012.12.005

accumbens after acute nicotine (Jackson et al., 2009d). Synapsin I mRNA is also increased in the locus coeruleus, amygdala, spinal cord, and pontine central gray area after chronic morphine treatment in rats (Matus-Leibovitch et al., 1995). Further, increased synapsin I phosphorylation and subsequent dopamine release were noted after amphetamine sensitization in rats, and after chronic amphetamine treatment in rat striatal synaptosomes (Iwata et al., 1996, 1997a, 1997b).

While these findings show that calcium-dependent mechanisms are relevant to drug-dependence behaviors, the changes occurring in post-receptor calcium-dependent signaling after chronic nicotine and withdrawal remain unclear. Thus, in the current study, we sought to complement previous behavioral findings from our lab indicating a role for calcium-dependent signaling in nicotine dependence and withdrawal (Damaj, 2005; Jackson and Damaj, 2009a) and expand on acute nicotine studies suggesting the importance of calcium-dependent mechanisms in mediating acute nicotine responses (Damaj, 2000, 2007; Jackson et al., 2009d) by examining CaMKII and synapsin I function in the nucleus accumbens after chronic nicotine exposure and withdrawal. Mice were chronically treated with nicotine for 14 days, and withdrawal was precipitated using the β2 nicotinic receptor-selective antagonist, dihydro-β-erythroidine (DHβE), and the α7 nicotinic receptor antagonist, methyllycaconitine citrate (MLA). A spontaneous withdrawal assessment was also conducted to complement our precipitated approach.

2. Materials and methods

2.1. Animals

Male adult C57BL/6 mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA). Animals were housed in a 21 °C humidity-controlled Association for Assessment and Accreditation of Laboratory Animal Care-approved animal care facility with food and water available ad libitum. The rooms were on a 12 h light/dark cycle (lights on at 7:00 A.M.). Mice were about 8–10 weeks of age and weighed approximately 25–30 g at the start of the experiment. All experiments were performed during the light cycle and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University.

2.2. Drugs

(-)-Nicotine hydrogen tartrate salt was purchased from Sigma Chemical Company (Milwaukee, WI). DHβE and MLA were purchased from RBI (Natick, MA, USA) and were dissolved in saline. Drugs were injected s.c. at a volume of 10 ml/kg body weight. The pH of the nicotine solution was checked and neutralized if necessary. All doses are expressed as the free base of the drug.

2.3. Chronic nicotine administration

Mice were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and implanted with Alzet osmotic minipumps [model 2002 (14 days); Durect Corporation, Cupertino, CA, USA] filled with (-)-nicotine or saline solution. The minipumps infused nicotine (36 mg/kg/day, s.c.) or saline for 14 days. The concentration of nicotine was adjusted according to animal weight and minipump flow rate.

2.4. Chronic nicotine and withdrawal studies

On the morning of day 15, male C57Bl/6 mice ($n=32$) were injected with vehicle or antagonist [DHβE (2 mg/kg, s.c.) or MLA (10 mg/kg, s.c.)]. Mice were sacrificed by cervical dislocation 20 min after antagonist injection. To assess protein levels after spontaneous withdrawal, in a separate group of male C57Bl\6 mice ($n=8$), minipumps were removed the afternoon of day 14. In brief, mice were anesthetized using isoflurane anesthesia. A small 5 mm incision was made on the skin surrounding the mini pump. The pump was removed through the incision, and the incision sutured with nylon monofilament. Mice were awake and active approximately 5 min after removal from isoflurane, and allowed to recover from surgery overnight. Mice were sacrificed the morning of day 15, approximately 24 h after cessation of nicotine treatment. For all studies, the brains were rapidly removed and sliced into 1 mm thick sections using a mouse brain matrix (Braintree Scientific Co., Braintree, MA, USA) on ice. The nucleus accumbens, consisting of both the shell and core divisions, was identified using a stereotaxic atlas (Paxinos and Franklin, 2001), dissected from the appropriate section (approximate coordinates Nucleus accumbens: Bregma 1.10 mm), and placed immediately in cold extraction buffer.

2.5. Western blot assays

Nucleus accumbens brain sections were homogenized in extraction buffer containing 50 mM Tris, 1% SDS, 1 mM PMSF, 1 mM EDTA, 5 mM EGTA, 1 mM Na^+ orthovanadate, 20 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 μM okadaic acid. Protein concentrations were determined using the Bradford assay. 30 μg of protein were incubated with 6X blue gel loading dye (New England Biolabs, Ipswich, MA, USA), and heated for 5 min at 95 °C. Samples were then separated by SDS-polyacrylamide gel electrophoresis on a 10% Tris–HCl gel and subjected to immunoblotting. Non-specific protein was blocked in 5% milk solution in TBS-T for 1 h at room temperature. Primary antibodies for α-CaMKII (1:1000; Sigma, St. Louis, MO, USA), α-pCaMKII (1:10000; Fisher Scientific, USA), synapsin I (1:2000; Chemicon International, Inc, Billerica, MA, USA), or pSynapsin I Ser603 (an antibody specific for the site phosphorylated by CaMKII) (1:2000; Sigma, St. Louis, MO, USA) and α-tubulin antibody (1:5000; Upstate, Temecula, CA, USA) were incubated overnight at 4 °C. Secondary antibodies (1:5000; LiCor Biosciences, Inc., Lincoln, NE, USA) were incubated for 1 h at room temperature the next day. Bound antibody was detected using the LiCor Odyssey Infrared Imaging System (LiCor Biosciences, Inc., Lincoln, NE, USA). α-CaMKII bands were detected at 50 kDa, α-pCaMK II bands were detected at 52 kDa, Synapsin I bands were detected at 80 kDa, pSynapsin I Ser603 bands were detected at 78 kDa, and α-tubulin bands were detected at 55 kDa. Blots were analyzed by taking the ratio of protein:α-tubulin. Results from two independent blots were combined, normalized, and represented as a percentage of saline baseline. The ratio of phosphorylated to total protein was also calculated using the formula [(phospho/α-tubulin)/(total/α-tubulin)].

2.6. Statistical analysis

For all data, statistical analyses were performed using StatView®. Data from precipitated western blot studies were analyzed using one-way analysis of variance with treatment as the between subject factor. Significant results were further assessed using a Neuman–Keuls post hoc test. For spontaneous nicotine withdrawal studies, data were analyzed using a student's unpaired *t*-test. *P*-values less than 0.05 were considered significant.

3. Results

3.1. DHβE, but not MLA, decreases total and phosphorylated CaMKII and synapsin I protein levels in the nucleus accumbens

Chronic nicotine infused for 14 days induced a significant increase in pCaMKII ($F_{(5,27)}=12.73$, $P<0.0001$) and CaMKII ($F_{(5,27)}=10.985$,