Neurobiology of Learning and Memory 106 (2013) 102–111

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Long term alterations in synaptic physiology, expression of β2 nicotinic receptors and ERK1/2 signaling in the hippocampus of rats with prenatal nicotine exposure

Kodeeswaran Parameshwaran [1], Manal A. Buabeid, Subhrajit Bhattacharya, Subramaniam Uthayathas [2], Thiruchelvam Kariharan, Muralikrishnan Dhanasekaran, Vishnu Suppiramaniam *

Department of Pharmacal Sciences, Harrison School of Pharmacy, Auburn University, Auburn, AL 36849, United States

ARTICLE INFO

Article history:
Received 24 April 2013
Revised 29 June 2013
Accepted 9 July 2013
Available online 18 July 2013

Keywords:
Synaptic plasticity
AMPA receptors
Nicotinic receptors
Glutamate
Single channel

ABSTRACT

Smoking during pregnancy is associated with long lasting, hippocampus dependent, cognitive deficits in children. The current study was performed to investigate the effect of prenatal nicotine exposure on excitatory synaptic physiology and cellular signaling in the hippocampus using a rodent model. Excitatory synaptic physiology was analyzed using electrophysiological methods to detect changes in synaptic plasticity, excitatory synaptic transmission and synaptic currents mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) in the hippocampus. Additionally, western blot experiments were performed to quantify alterations in protein expression levels in the hippocampus. Prenatal nicotine exposure resulted in a decrease in long term potentiation (LTP) and an increase in long term depression (LTD). Basal synaptic transmission was also reduced with a concomitant decline in AMPAR mediated synaptic currents at the cellular and single channel levels. Presynaptic pool of vesicles docked close to release sites were also diminished in nicotine exposed rats. Moreover, reduced levels of β2 subunit containing nicotinic receptors and extracellular signal regulated kinase1/2 (ERK1/2) were observed in nicotine exposed rats. These results suggest that long lasting alterations in excitatory synaptic physiology, AMPAR synaptic currents and ERK1/2 signaling may serve as the molecular mechanisms for cognitive deficits associated with prenatal nicotine exposure.

1. Introduction

Nicotinic acetylcholine receptors (nAChRs) represent a group of pentameric ligand gated ion channels that generate excitatory neurotransmission when activated. These receptors are widely distributed in the mammalian central nervous system and perform essential functions, including regulating the activity of other neurotransmitter systems (Dani & Bertrand, 2007). During brain development the nAChRs are expressed relatively early and reach their maximal expression levels (Adams et al., 2002). Developmental roles of nAChRs encompass spatio-temporally defined regulation of neuronal morphogenesis, cell survival and cell death (Ballesteros-Yanez, Benavides-Piccione, Bourgeois, Changeux, & DeFelipe, 2010; Hory-Lee & Frank, 1995; Lauder & Schambra, 1999; Orr-Urtreger et al., 2000). In the developing hippocampus nAChRs mediate spontaneous excitatory neuronal firings. Such neuronal firings propagating as wave-like activation oscillations were also observed in other developing regions of central nervous system (Bansal et al., 2000; Feller, 1999; Hanson & Landmesser, 2003). During development, presynaptic modulation by nAChRs activates the silent synapses in the hippocampus and thereby strengthens the glutamatergic transmission (Maggi, Le Magueresse, Changeux, & Cherubini, 2003). In summary, these reports highlight the essential roles of nAChRs on development and excitatory synaptic physiology in the hippocampus.

Abnormal nAChR activity during neurodevelopment can, therefore, cause enduring changes in physiological and micro anatomical processes regulated by these receptors. Such abnormal activation of nAChRs occurs in cases of tobacco use (primarily in the form of smoking) during pregnancy. Smoking during pregnancy is quite high with about 20% of women aged between 18 and 44 years reported smoking, making the estimates to 1 in 7 pregnancies in the United States (Davis et al., 2009; Tong, Jones, Dietz, D'Angelo, & Bombard, 2009; Ward, Lewis, & Coleman, 2007; Zhao et al., 2012). Several clinical reports had established that cigarette use during pregnancy is a high risk factor for adverse health conditions in children (Blood-Siegfried & Rende, 2010;

* Corresponding author. Fax: +1 334 844 8331.
E-mail address: suppivd@auburn.edu (V. Suppiramaniam).
[1] Present address: Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, United States.
[2] Present address: Department of Pharmacology, College of Medicine, Howard University, Washington, DC 20059, United States.

1074-7427/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.nlm.2013.07.007

Ernst, Moolchan, & Robinson, 2001; Murin, Rafii,& Bilello, 2011; Pavic, Dodig, Jurkovic, Krmek, & Spanovic, 2011; Ruckinger et al., 2010; Rydell, Cnattingius, Granath, Magnusson, & Galanti, 2012). In particular, deficits in tasks that require learning, memory, and problem solving skills are impaired in children following exposure to gestational smoke exposure (Cornelius, Ryan, Day, Goldschmidt, & Willford, 2001; DiFranza, Aligne, & Weitzman, 2004). Many of the behavioral consequences, including cognitive impairments, can be long lasting (Murin et al., 2011; Weissman, Warner, Wickramaratne, & Kandel, 1999), which mandate the need for better understanding of the underlying pathophysiology of prenatal nicotine exposure.

Previously, we reported that in rats prenatal nicotine exposure resulted in mood disorders, deficits in spatial memory, reduced LTP in the hippocampus, and decreased basal synaptic transmission and synaptic currents gated by AMPARs (Parameshwaran et al., 2012). The processes that impose enduring changes in cognition need further investigation. Hence, in this study, we have performed LTP and LTD experiments in two months old rats to investigate the long-lasting effects of prenatal nicotine exposure on synaptic plasticity. Basal synaptic transmission, presynaptic release patterns and synaptic currents mediated by AMPARs in the hippocampus were also analyzed. These extensive electrophysiological studies were complemented with the measure of protein levels to detect changes in β2 subunit containing nAChRs and ERK1/2, a member of mitogen activated protein kinases (MAPK).

2. Materials and methods

2.1. Animals and chemicals

Osmotic mini pumps (Alzet, Cupertino, CA), filled with sterile physiological saline or nicotine, were implanted beneath the shoulder skin of anesthetized timed pregnant Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) to deliver a subcutaneous dose of saline or nicotine (free base diluted with sterile physiological saline at a rate of 6 mg/kg/day). On the day of parturition, after all pups were born, osmotic mini pumps were removed under brief isoflurane anesthesia, and the wounds were closed with surgical clips. This ensured that the nicotine exposure was prenatal and spanned from day-3 of pregnancy to birth. The day-after parturition was considered day 1 (PND 1) and litters were culled to ten pups per dam with, if possible, an equal sex ratio. Rats were randomly chosen among different litters for the experiments. Rats were housed under standard conditions (12 h light/dark cycle with access to food and water *ad libitum*). All the experimental procedures involving live animals were performed as per the NIH guidelines and the protocol approved by the Auburn University Institutional Animal Care and Use Committee. Unless specified, all the chemicals were purchased from Sigma (St. Louis, MO).

2.2. Preparation of hippocampal slices and synaptosomes

Transverse hippocampal slices (400 μm) were prepared as described previously (Parameshwaran et al., 2007; Parameshwaran et al., 2012). In brief, hippocampal slices were prepared while immersed in ice cold dissection buffer containing (in mM): 85 NaCl, 2.5 KCl, 4 $MgSO_4$, 0.5 $CaCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 0.5 ascorbate and 2 kynurenic acid; bubbled with 95%CO_2/5%O_2, pH 7.4. The slices were incubated for 1 h in artificial cerebrospinal fluid (aCSF) containing (in mM): 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 1 NaH_2PO_4, 26 $NaHCO_3$ and 11 dextrose; bubbled with 95%CO_2/5%O_2. Synaptosomes were prepared by previously described methods (Johnson, Chotiner, & Watson, 1997; Suppiramaniam, Vaithianathan, & Parameshwaran, 2006) in which hippocampi were dissected out and homogenized in a buffer (mKRBS) consisted of (in mM): 118.5 NaCl, 4.7 KCl, 1.18 $MgSO_4$, 2.5 $CaCl_2$, 1.18 KH_2PO_4, 24.9 $NaHCO_3$, 10 dextrose and 10 mg/ml adenosine deaminase. The pH was adjusted to 7.4 by bubbling with 95%O_2/5%CO_2. The buffer was also supplemented with 0.01 mg/ml leupeptin, 0.005 mg/ml pepstatin A, 0.10 mg/ml aprotinin and 5 mM benzamide to minimize proteolysis. The homogenate was filtered twice and centrifuged at 1000*g* for 15 min at 4 °C. The supernatant was removed, and the pellets, which contained synaptosomes, were resuspended in mKRBS buffer.

2.3. Slice electrophysiology

Following incubation electrophysiological recordings were performed in a recording chamber with continuous perfusion of aCSF bubbled with 95%CO_2/5%O_2. Field excitatory postsynaptic potentials (fEPSP) from Schaffer collateral/commissural-CA1 synapses were recorded by stimulating CA1 stratum radiatum with bipolar electrodes and placing a recording glass electrode (1–4 MΩ) filled with aCSF ~ 200 μm from the stimulating electrode. The frequency of the test stimulation was 0.33 Hz. For stimulus response curves current intensity was increased from 0 to 300 μA at steps of 25 μA. For LTP and presynaptic vesicle draining experiments current intensity was set at 50% of the amplitude at which the initial population spikes began to appear. In LTP experiments after at least 15 min of stable baseline recording 3 high frequency stimuli (HFS; 100 pulses, 100 Hz) were delivered every 20 s. LTP was measured 55–60 min post HFS. LTD was induced by two low frequency stimuli (LFS; 900 pulses at 1 Hz) separated by 10 min and preceded by 15 min of baseline recordings. LTD was measured at 55–60 min from the end of second LFS. Stimulation intensity was set at ~60% (during LFS) or 40% (all other times excluding LFS) of the amplitude at which initial population spikes began to appear.

Whole cell current recordings were performed in CA1 pyramidal neurons voltage clamped at −80 mV. The patch pipette (6–10 MΩ) was filled with an internal solution comprising (in mM): 100 K-gluconate, 0.6 EGTA, 5.0 $MgCl_2$, 2.0 Na-ATP, 0.3 Na-GTP and 40 HEPES; pH 7.4. The slices were perfused continuously with oxygen saturated aCSF that was supplemented with picrotoxin (50 μM), DL-2-amino-5-phosphonopentonoic acid (100 μM) and tetrodotoxin (1 μM) to pharmacologically isolate AMPAR mediated miniature excitatory postsynaptic currents (mEPSC). Currents were low-pass filtered (2 kHz), digitized (10 kHz), amplified (Axopatch 200B, Molecular Devices), and acquired with the pCLAMP 8 program (Molecular Devices). To be included for analysis the holding currents should be greater than the baseline noise levels but less than −150 pA and series resistance was in the range of ~20–30 MΩ and did not drift by >15%. Data were analyzed with the Mini Analysis program (Synaptosoft, Fort Lee, NJ) and the mEPSCs included for analysis had a monotonic 10–90% rise time of <6 ms and exponential decay time <25 ms. The vast majority of the mEPSCs had rise times and decay times that were considerably less than these upper limits. The mEPSC amplitude (A) was measured from the baseline with the amplitude threshold for detection of mEPSCs was set above the noise level, at four times the SD of mean noise level, and individual events were confirmed visually.

2.4. Single channel electrophysiology

Incorporation of AMPARs from synaptosomal fractions in artificial lipid bilayers was carried out using 'tip-dip" method (Suppiramaniam et al., 2006; Vaithianathan et al., 2005). In brief, a phospholipid bilayer was formed at the tip of a polished borosilicate glass pipette (100 M) filled with intracellular solution. The