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Research Paper

Influence of developmental nicotine exposure on glutamatergic neurotransmission in rhythmically active hypoglossal motoneurons

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ABSTRACT

Developmental nicotine exposure (DNE) is associated with increased risk of cardiorespiratory, intellectual, and behavioral abnormalities in neonates, and is a risk factor for apnea of prematurity, altered arousal responses and Sudden Infant Death Syndrome. Alterations in nicotinic acetylcholine receptor signaling (nAChRs) after DNE lead to changes in excitatory neurotransmission in neural networks that control breathing, including a heightened excitatory response to AMPA microinjection into the hypoglossal motor nucleus. Here, we report on experiments designed to probe possible postsynaptic and presynaptic mechanisms that may underlie this plasticity. Pregnant dams were exposed to nicotine or saline via an osmotic mini-pump implanted on the 5th day of gestation. We used whole-cell patch clamp electrophysiology to record from hypoglossal motoneurons (XIIMNs) in thick medullary slices from neonatal rat pups ($N = 26$ control and 24 DNE cells). To enable the translation of our findings to breathing-related consequences of DNE, we only studied XIIMNs that were receiving rhythmic excitatory drive from the respiratory central pattern generator. Tetrodotoxin was used to isolate XIIMNs from presynaptic input, and their postsynaptic responses to bath application of L-glutamic acid (glutamate) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) were studied under voltage clamp. DNE had no influence on inward current magnitude evoked by either glutamate or AMPA. However, in cells from DNE animals, bath application of AMPA was associated with a right shift in the amplitude distribution ($P = 0.0004$), but no change in the inter-event interval distribution of miniature excitatory postsynaptic currents (mEPSCs). DNE had no influence on mEPSC amplitude or frequency evoked by glutamate application, or under (unstimulated) baseline conditions. Thus, in the presence of AMPA, DNE is associated with a small but significant increase in quantal size, but no change in the probability of glutamate release.

1. Introduction

According to the Centers for Disease Control (CDC) cigarette smoking is one of the leading causes of preventable disease in the United States, resulting in 1 out of 5 deaths annually (www.cdc.gov/tobacco). Nicotine is the primary neurotoxin in tobacco products (Abreu-Villaca et al., 2003; Eriksson et al., 2001; Ginzel et al., 2007; Pauly and Slotkin, 2008), as it has been strongly associated with altered development of neurons throughout the brain (Eriksson, 1997; Slotkin, 2004), including brainstem neurons involved in the control of breathing (Dwyer et al., 2009; Hafstrom et al., 2005; Muhammad et al., 2012; Pilarski et al., 2011). Despite an overall decline in the use of cigarettes in the U.S. in the last decade, the incidence of nicotine use during gestation remains unaddressed, as nicotine replacement therapy (patch, gum) is commonly prescribed to pregnant women who continue to smoke (Oncken, 2012). Developmental nicotine exposure (DNE) in rodents is a widely accepted model for studying the effects of in utero exposure to nicotine (Slotkin, 2004). Chronic nicotine exposure causes widespread desensitization of nicotinic acetylcholine receptors (nAChRs), leading to increased receptor expression, but a reduction in synaptic efficacy (Gentry and Lukas, 2002; Marks et al., 1983, 1985). This paradox results in a "functional loss" of nAChRs and is due to long-term receptor desensitization. Consistent with this, our previous work (Pilarski et al., 2012) showed that DNE caused functional desensitization of nAChRs on hypoglossal motoneurons (XIIMNs). Hypoglossal motoneurons control the activity of the tongue muscles, which play an important role in swallowing and chewing, as well as breathing, especially during sleep (Eisele et al., 2003; Kezirian et al., 2010), and excitatory drive to XIIMNs relies importantly on glutamatergic neurotransmission (Revill et al., 2015; Steenland et al., 2006).

Our laboratory previously reported that DNE decreased excitatory synaptic input in XIIMNs of neonatal rats (Pilarski et al., 2011), and increased the response of XIIMNs to microinjection of glutamate or AMPA into the hypoglossal motor nucleus (Jaiswal et al., 2013). These observations lead to the development of a model that is based on the working hypothesis that the reduced presynaptic excitatory input that

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occurs as a result of nAChR desensitization leads to an increased density, and/or a functional change, in postsynaptic glutamate receptors, as summarized in Fig. 1. Here we focus on two components of this model, namely postsynaptic changes in glutamatergic transmission, and alterations in the quantity and frequency of glutamate release from glutamatergic neurons that synapse on XIIMNs.

We used the whole-cell patch-clamp technique to record from rhythmically-active XIIMNs in thick medullary slices. Postsynaptic effects were examined by bath applying glutamate or AMPA in the presence of tetrodotoxin (TTX) and recording the kinetics and magnitude of the evoked inward current. We also examined changes in quantal synaptic transmission by recording the frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs), under baseline conditions and also during bath application of glutamate or AMPA. In the presence of AMPA, mEPSC amplitude was larger in DNE than in control and their frequency greater, consistent with an increase in quantal size and an increase in the probability of glutamate release; mechanisms that may explain these observations are addressed in the Discussion.

2. Materials and methods

2.1. Animals

We used a total of 50 Sprague-Dawley rats of either sex, aged from postnatal day zero (P0) through postnatal day four (P4). This neonatal period corresponds to a gestational age of 29–34 weeks in humans (Ballanyi et al., 1999). The nicotine-exposed neonates were taken from 19 separate litters; the saline-exposed neonates from 15 different litters; and the unexposed from 3 litters. From each litter, 1–3 neonates were used. All neonates were born via spontaneous vaginal delivery from pregnant adult female rats purchased from Charles River Laboratories (Wilmington, MA). Neonatal rat pups were housed together with their mothers and siblings until they were studied. Dams were housed in the Animal Care Facility at the University of Arizona under a 12: 12 h light/dark cycle (lights on 07:00 h) with water and food available ad libitum, in a quiet room at 22 °C and 20–30% relative humidity. All protocols were approved by the University of Arizona Institutional Care and Use Committee and conformed to National Institutes of Health guidelines.

2.2. Developmental nicotine exposure

DNE was achieved by subcutaneous implantation of Alzet 1007D mini-osmotic pumps (Alzet Corp., CA, USA) into pregnant dams on gestational day 5 under aseptic conditions, as previously described (Fregosi et al., 2004; Huang et al., 2004; Luo et al., 2007). The pumps allow for fluid infusion into the subcutaneous space at a rate of 2.5 μL h^{-1} for 28 days. This infusion rate achieves mean nicotine bitartrate delivery of 6 mg kg^{-1} day^{-1} throughout gestation. We previously showed that this regimen produces plasma levels of cotinine, a major metabolite of nicotine with a long half-life (20–24 h), ranging from 60 to 92 ng/mL (Powell et al., 2015). This is comparable to average cotinine levels (88 ng/mL) found in the umbilical cord blood of newborns whose mothers smoked on average 95 cigarettes/week (Berlin et al., 2010).

2.3. Medullary slice preparation

Pups of either sex were removed from their home cages at random and weighed. Animals were anesthetized by hypothermia until unresponsive to a paw pinch, and decerebrated at the coronal suture. The vertebral column and ribcage were exposed, moved to a dissection dish filled with cold (4–8 °C) oxygenated (95% O_2–5% CO_2) artificial cerebrospinal fluid (aCSF), composed of (in mM): 120 NaCl, 26 $NaHCO_3$, 30 glucose, 1 $MgSO_4$, 3 KCl, 1.25 NaH_2PO_4, and 1.2 $CaCl_2$ with pH adjusted to 7.4 and osmolarity adjusted to 300–325 mOsm. The spinal cord and brainstem were glued to an agar block with superglue, rostral surface up, for serial microsectioning in a vibratome (VT1000; Leica). Transverse medullary slices were taken in chilled oxygenated aCSF until the most rostral hypoglossal nerve (XIIn) rootlets were near the surface of the tissue. A single 700-μm slice was taken to capture the majority of hypoglossal motor nucleus and the preBotzinger complex,

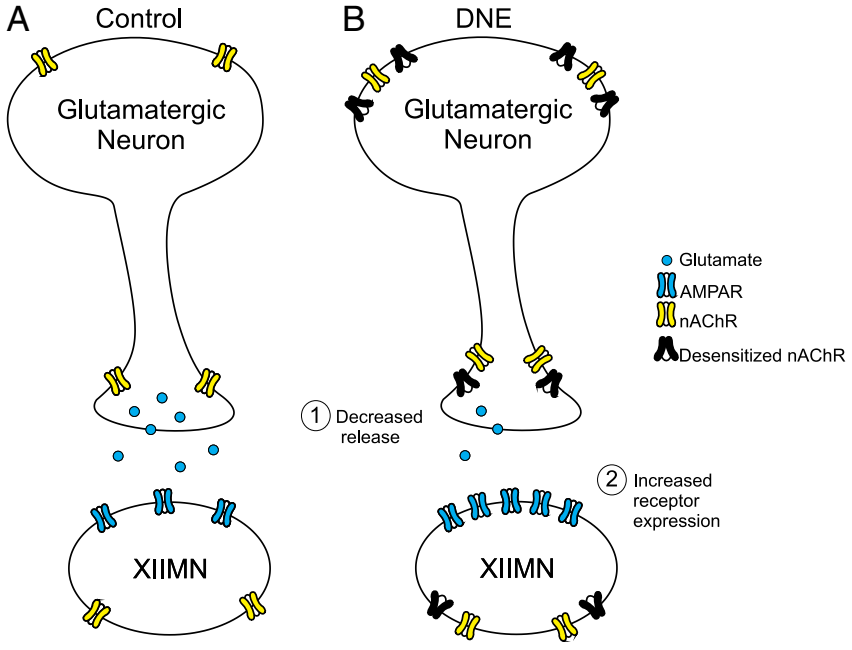

Fig. 1. Diagram illustrating possible mechanisms of DNE-induced plasticity of glutamatergic neurotransmission in XIIMNs. (A) The nAChRs on glutamatergic neurons are located on the soma, and also on the presynaptic terminals. When nicotine or ACh binds to nAChRs on the soma of the glutamatergic neuron, sufficient depolarization can evoke action-potential-dependent release of glutamate. When terminal nAChRs are stimulated by nicotine or ACh, vesicular release of glutamate is increased secondary to a rise in intracellular calcium; this reinforces the random, spontaneous vesicular release of glutamate. The XIIMNs express both nAChRs and AMPA receptors. (B) DNE is associated with an increase in nAChRs on both the cell body and terminals, but many of these receptors are in a desensitized state. As explained in the text, the net effect of DNE is a "functional loss" of nAChR-mediated synaptic transmission. This functional loss could lead to both presynaptic (decreased glutamate release) and postsynaptic (upregulation of glutamate receptor expression) plasticity.