Neuropharmacology 137 (2018) 71–85

Contents lists available at ScienceDirect

Neuropharmacology

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

Prenatal nicotine exposure alters postsynaptic AMPA receptors and glutamate neurotransmission within the laterodorsal tegmentum (LDT) of juvenile mice

Filip S. Polli, Kristi A. Kohlmeier*

Department of Drug Design and Pharmacology, Faculty of Health Sciences, University of Copenhagen, Copenhagen 2100, Denmark

ARTICLE INFO

Article history:
Available online 25 April 2018

Keywords:
Prenatal nicotine
AMPA receptor
Patch clamp
Calcium imaging
Laterodorsal tegmental nucleus

ABSTRACT

Despite dissemination of information regarding the harm on fetal development of smoking while pregnant, the number of pregnancies associated with nicotine exposure appears to have stagnated. Presence of nicotine during neural formulation is associated with a higher susceptibility of drug dependence, suggesting an altered development of neurons in circuits involved in saliency and motivation. The laterodorsal tegmental nucleus (LDT) plays a role in coding stimuli valence via afferents to mesolimbic nuclei. Accordingly, alterations in development of neural mechanisms in the LDT could be involved in vulnerability to drug dependency. Therefore, we examined the effect of prenatal nicotine exposure (PNE) on glutamatergic functioning of LDT neurons in mouse brain slices using whole-cell, patch clamp concurrent with fluorescence-based calcium imaging. PNE was associated with larger amplitudes of AMPA-induced currents, and greater AMPA-mediated rises in intracellular calcium. AMPA/NMDA ratios and the AMPA-current rectification index were lower and higher, respectively, consistent with changes in the functionality of AMPA receptors in the PNE, which was substantiated by a greater inhibition of evoked and spontaneous glutamatergic synaptic events by a selective inhibitor of GluA2-lacking AMPA receptors. Paired pulse ratios showed a decreased probability of glutamate release from presynaptic inputs, and fluorescent imaging indicated a decreased action potential-dependent calcium increase associated with PNE. When taken together, our data suggest that PNE alters LDT glutamatergic functioning, which could alter output to mesolimbic targets. Such an alteration could play a role in altered coding of relevancy of drug stimuli that could enhance risk for development of drug dependency.

1. Introduction

Around the world, public health sectors have employed extensive efforts to inform society of the deleterious effects of smoking during pregnancy. In addition, governmental control has been exerted over the tobacco industry to impact tobacco use via implementation of high taxes and advertisement bans. Despite these prevention attempts, many women continue to smoke, and a considerable number of children are born from women who smoked during their pregnancy. Further, in a well-intentioned action elicited to avoid harmful effects of tobacco products, many women switch during their pregnancy to non-combustible methods of nicotine exposure. This change in behavior remains problematic as these alternative products do still contain nicotine, the psychobiologically-active compound which engenders continued use. Nicotine is quickly absorbed by the circulatory system of the mother and easily crosses the placenta where it accumulates in fetal compartments at a higher concentration when compared with levels in maternal tissue (Luck et al., 1985; Slotkin, 1998). Nicotine is a teratogen and accordingly, its actions have been shown to alter the developing brain, and it is these drug-induced alterations which likely result in neurally-based, adverse behavioral outcomes for prenatally exposed offspring (Holbrook, 2016). One adverse behavioral outcome for those prenatally exposed to nicotine is their vulnerability to the development of addictive behaviors, especially during the developmental period prior to adulthood. A growing number of cohort-based studies have reported that prenatal cigarette exposure is associated with nicotine dependence later in life during both adolescent and adult periods

* Corresponding author. Department of Drug Design and Pharmacology, Faculty of Health Sciences, Universitetsparken 2, University of Copenhagen, Copenhagen, 2100, Denmark.
E-mail address: kak1@sund.ku.dk (K.A. Kohlmeier).

https://doi.org/10.1016/j.neuropharm.2018.04.024
0028-3908/

(Buka et al., 2003; Cornelius et al., 2012; O'Callaghan et al., 2009; Rydell et al., 2012; Shenassa et al., 2015). Although confounds exist, this association was found in studies in which postnatal cigarette parental smoking bias was controlled (De Genna et al., 2016). Elevated risks for other drug experimentation were also reported (Lotfipour et al., 2014). Higher risk could be due mechanistically to demonstrated alterations of development in prenatally exposed individuals of neural circuits that are involved in encoding incentive salience critical in the process of development of dependency to drugs of abuse (for review, see Kohlmeier, 2015; Müller et al., 2013).

Addiction-promoting stimuli, such as nicotine, are given value, or incentivized, by being perceived as a reward via their ability to trigger large rises of dopamine (DA) from neurons of the ventral tegmental area (VTA) into the nucleus accumbens (NAc). Large DA effluxes are associated with a switch from a tonic pattern to a burst-firing pattern. Burst-firing sufficient to result in behaviorally-relevant patterns of release of DA from the VTA was found to depend on an intact laterodorsal tegmental nucleus (LDT). The LDT is a nucleus in the pons which is comprised of cholinergic, GABAergic and glutamatergic neurons (Lodge and Grace, 2006; Omelchenko and Sesack, 2005; Wang and Morales, 2009). Drugs of abuse can exert actions directly in the VTA resulting in stimulation of DA release; however, indirect stimulation of the VTA could occur via activation of the LDT, as neurons of the LDT have been shown to be directly activated by several drugs of abuse. Acute application of nicotine activates pre and postsynaptic nAChRs in the LDT, which would be expected to alter output to the VTA (Ishibashi et al., 2009), and *ex vivo* cocaine exposure elicits rises in intracellular calcium within LDT neurons (Lambert et al., 2017). In addition, intraperitoneal administration of methamphetamine increases acetylcholine levels within the VTA of mice. This effect was attenuated by intra-LDT bilateral microinjections of the muscarinic 2 agonist, oxotremorine (Dobbs and Mark, 2012; Laviolette et al., 2000). Interestingly, chronic exposure to drugs of abuse has been shown to induce changes within the LDT. Greater excitability was found in the LDT-VTA-NAc pathway after chronic exposure to amphetamine. This finding suggested that the LDT might contribute to long-term potentiation (LTP) occurring at excitatory synapses onto DA VTA neurons known to be relevant for behavioral sensitization (Nelson et al., 2007). Further, repeated cocaine exposure in rats led to increased amplitude and frequency of miniature excitatory synaptic currents (mEPSCs), as well as reduced paired pulse ratios (PPR) in LDT neurons (Kurosawa et al., 2013). Taken together, these findings place the LDT within the circuitry underlying the neurobiology of addiction and highlight that drugs of abuse can alter the function of neurons in this nucleus.

Since the LDT plays a role in the cellular mechanisms responsible for assignment of saliency to stimuli, it is important to determine how gestational nicotine impacts neurons in the LDT if we are to understand the neuronal underpinnings of the higher liability to addiction behavior exhibited by this population. We have shown previously that gestational exposure to nicotine alters cellular responses of LDT neurons to excitatory input impinging on acetylcholine receptors. Nicotine induces smaller intracellular calcium rises and larger amplitudes of excitatory synaptic currents (sEPSCs) in prenatally nicotine exposed (PNE) young mice when compared with matched controls. Kinetic changes in the action potential were also observed (Christensen et al., 2015). Furthermore, although we did not identify the underlying mechanism, we have shown that PNE is associated with reduced AMPA and NMDA receptor-mediated intracellular rises in calcium in LDT neurons (McNair and Kohlmeier, 2015). As alterations in glutamatergic mechanisms play a key role in addiction-related neurobiology, we wished to extend our findings by determining whether PNE is associated with persistent cellular and molecular changes in glutamatergic signaling within the LDT of postnatal mice. Further, we wished to examine the potential functional consequence of alterations in glutamatergic excitability within LDT synapses. When taken together, the results from our present study provide further evidence that nicotine during pregnancy induces alterations in excitatory transmission. These changes include a heightened GluA2-lacking AMPAR subunit function and decreased glutamate neurotransmission within LDT neurons. Alterations in glutamate signaling in the LDT would be expected to impact output to target regions, which could play a role in the coding of motivational and arousing stimuli.

2. Methods

2.1. Animals

Animal procedures were authorized by the Animal Welfare Committee appointed by the Danish Ministry of Justice and in accordance with Danish and European directives for the usage of rodents in research. Our laboratory has valid permits to utilise the prenatal nicotine exposure model (PNE) and to employ all procedures described below (2012-15-2934-0033 and 2012-15-2934-0037). All the procedures performed were conducted by trained personal and animal suffering was minimized whenever possible. NMRI mice (Taconic, Denmark) aged between 10 and 21 days were used for electrophysiology and calcium imaging experiments. The mice were kept under standard conditions, 12 h light/dark cycle, controlled humidity (36–58%), constant temperature ($22 \pm 2\,^{\circ}C$) and with *ad libitum* access to food and water. Naïve female adult mice were individually housed and received either distilled water flavored with 2% saccharine or the same saccharine solution with the addition of nicotine through the drinking solution (week −1). A male mouse was added to the cage containing the female (week 0) on the second week after the female started receiving the solutions in both groups to induce pregnancy (week 1 and week 2). The males were removed when the dams were visibly pregnant. Pregnant females drank from the saccharine or nicotine solutions until the birth of their offspring. Offspring between the ages of 10 and 21 days were used, and potential confounds of the estrus cycle were eliminated by using male offspring for all experiments.

2.2. Prenatal nicotine exposure model

Female mice exposed to nicotine received 300 μg/mL of nicotine through their drinking water during their entire pregnancy, as previously published (Christensen et al., 2014; Pauly et al., 2004). This model has been shown to result in molecular and behavioral changes in pups indicative of nicotine exposure *in utero* (Alkam et al., 2013; Christensen et al., 2015; Heath et al., 2010; Schneider et al., 2011). Interestingly, in pregnant women who smoke, it has been shown that nicotine accumulates in the placenta and amniotic fluid, significantly increasing the concentration of nicotine experienced by the fetus when compared to the blood nicotine concentration of the mother (Luck et al., 1985). As further support of the oral delivery of nicotine rodent model, while dose responses were noted in the levels of cotinine in the plasma of dams, there were not significant differences in levels noted in pups at nicotine concentrations exceeding 100 μg/mL (Pauly et al., 2004). To increase palatability, saccharine was added to the solution, and the pH was adjusted to 7.8 with chloride acid (1 M). The solution intake was measured and replaced twice per week. In our study, nicotine treated dams did drink significantly less solution than controls across all measurements during treatment (Table 1), which is a finding reported by others (Pauly et al., 2004; Schneider et al.,