Neurobiology of Learning and Memory 90 (2008) 527–536

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Long-lasting teratogenic effects of nicotine on cognition: Gender specificity and role of AMPA receptor function

J. Vaglenova *, K. Parameshwaran, V. Suppiramaniam, C.R. Breese, N. Pandiella, S. Birru

Department of Pharmacal Sciences, Harrison School of Pharmacy, Auburn University, Auburn, Al 36849, USA

ARTICLE INFO

Article history:
Received 2 November 2007
Revised 20 June 2008
Accepted 30 June 2008
Available online 13 August 2008

Keywords:
Prenatal nicotine
Learning and memory
Avoidance
Anxiety
AMPA receptors

ABSTRACT

Nicotine, the main psychoactive ingredient in tobacco, readily crosses the placental barrier to cause growth and neurobehavioral abnormalities in the offspring. The current study was designed to assess whether nicotinic action causes long lasting teratogenic effects and synaptic dysfunctions. Pregnant Sprague–Dawley rats were infused with nicotine via osmotic minipumps at a dose of 6 mg/kg/day corresponding to the dose receiving during heavy smoking. A battery of behavioral tests and electrophysiological experiments were performed during specific postnatal periods. A spectrum of developmental and behavioral modifications in adolescent, young-adult and aged animals resulted after prenatal nicotine exposure. The potentially teratogenic effect of nicotine was clearly demonstrated in both genders by changes in developmental reflexes, exploratory and novelty seeking behavior, as well as a higher level of anxiety, and changes in individual and group responses in learning and memory. Most of the behavioral abnormalities were transitional with advancing age (6 months), although cognitive deficits measured by a two-way active avoidance task were long-lasting for male rats. Electrophysiological studies show decreased excitatory postsynaptic responses (mEPSCs) mediated by AMPA receptors in the hippocampus. These results suggest that teratogenic effect of nicotine on cognition is age and gender-specific, long-lasting and associated with AMPA receptor function.

1. Introduction

Nicotine, the main psychoactive ingredient in tobacco, readily crosses the placental barrier to cause growth and neurobehavioural abnormalities in the offspring. Animal models are critical for corroborating human studies and subcutaneous infusion of nicotine via osmotic mini pumps in rodents is a preferred method of exposure because of the minimal side effects and possibility of duplicating nicotine levels similar to those reported in human cases (Murrin, Ferrer, Zeng, & Haley 1987). In a previous study Vaglenova, Birru, Pandiella, and Breese (2004) analyzed developmental, behavioral, and cognitive deficits following full-term prenatal nicotine exposure (from gestation day 3rd to the birth) at a rate of 6 mg/kg/day in rats. The administration model produced maternal plasma nicotine levels corresponding to those seen in heavy smokers (see Slotkin, Ryde, Tate, & Seidler, 2007). In another dose-dependent study only the dose of 6 mg/kg of nicotine caused growth retardation and deviations in the exploratory activity, higher anxiety levels in adolescence, and learning and memory deficits in the young-adult offspring of both genders (Vaglenova, Birru, & Breese, 2004b). The cross-fostering method also revealed that maternal behavior and nicotine withdrawal did not affect postnatal somatic retardation or cognitive abilities (Vaglenova et al., 2004a).

In some studies, motor, sensory, and cognitive deficits in infants and toddlers exposed prenatally to tobacco (Ernst, Moolchan, & Robinson, 2001; Gusella & Fried, 1984; Lichtensteiger, Ribary, Schlumpf, Odermatt, & Widmer, 1988; Olsen, 1992; Saxton, 1978) have been apparent at least up to 1 year of age. Other studies have suggested that behavioral and cognitive deficits may continue into late childhood and adolescence (DiFranza & Lew,1995; Milberger, Biederman, Faraone, & Jones, 1998; Naeye & Peters, 1984; Niaura et al., 2001; Orlebeke, Knol, & Verhulst, 1999; Wakschlag et al., 1997). Longitudinal studies remain controversial because they are not consistent in their reporting of the degree of recovery of these children to normal intellectual level. Some data, however, showed that the effects of teratogenic agents are modifiable with age, and indeed the affected subjects might respond to treatment (Milner, Squire, & Kandel, 1998; Vaglenova et al., 2008; Vaglenova & Petkov 1998; Vaglenova & Vesselinov Petkov, 2001).

The present study was designed to analyze deficits caused by prenatal nicotine exposure at a rate of 6 mg/kg/day throughout the pregnancy, on a well-defined experimental model prepared according to the guidance for the investigation of the drugs' ter-

* Corresponding author. Fax: +1 419 434 4390.
E-mail address: vagleju@yahoo.com (J. Vaglenova).

1074-7427/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.nlm.2008.06.009

atogenic effects (Ulbrich & Palmer, 1996). The model is indicative for cognitive and motor functions, anxiety and hippocampal AMPAR mediated synaptic transmission in rats. We predicted that cognitive decrements found in young adult offspring would last throughout the entire lifespan. Recent findings at the molecular levels support this hypothesis, showing long-lasting and sex-selective reprogramming in acetylcholine and serotonin synaptic neurotransmitter activity, throughout adenylyl cyclase (Slotkin, MacKillop, et al., 2007; Slotkin et al., 2007). To our knowledge there is still no substantial experimental evidence supporting the assumption that nicotine's teratogenic effects on cognition are long-lasting with advancing age, however. Therefore, we chose to study nicotine's teratogenic effects on six-month-old rats because cognitive deficits caused by prenatal drug exposure could be masked by natural amnesic events at a later age. Learning task performance begins to decline as early as middle age in both humans and rats (Woodruff-Pak, Jaeger, Gorman, & Wesnes, 1999). Another reason to investigate the rats at this age is the nature of the associative learning task used in our study, which mimics human cognitive processes. The task requires the participation of audiovisual analyzers, and specific paths and structures of CNS as cortex and limbic system. A limited electrical shock was used as unconditional stimulus and visual and auditory cues were used as conditional stimuli. Recent data indicate that aging animals are resistant to both the negative and the positive consequences of the stressful experience (Shors, 2006) that contribute to associative learning.

The previous study (Vaglenova et al., 2004a) did not directly address the explicit site of the neuropathological lesion induced by prenatal nicotine. Without disregarding studies of other neurotransmitter systems showing persistent and late-emerging, sex-selective deficits (Slotkin, MacKillop, et al., 2007; Slotkin et al., 2007), we focused our attention on the functioning of glutamatergic transmitter systems, especially AMPA receptors' system. The process of cognition is complex, involving many brain structures and systems. Increasing evidence indicates that the hippocampus, one of the main structures of the limbic system, plays a critical role in learning and memory (Bruel-Jungerman, Rampon, & Laroche, 2007; Martin & Clark, 2007).

In the hippocampus glutamatergic synaptic transmission mediates major excitatory neurotransmission and plays a vital role in synaptic plasticity that underlies learning and memory encoding (Baudry & Lynch, 1981; Kullmann & Lamsa, 2007; Miyamoto, 2006; Sprengel, 2006). In particular, AMPARs are critical for maintenance of long-lasting enhancements to synaptic plasticity (Fitzjohn., Doherty, & Collingridge, 2006; Malinow & Malenka, 2002; Sprengel, 2006). Recently Vaglenova et al. (2008) and Wijayawardhane et al. (2007) established that prenatal ethanol exposure may result in reduced AMPA-receptor functioning, suggesting that similar effects may following exposure to other harmful drugs during CNS development. We investigated the miniature excitatory postsynaptic currents mediated by AMPA receptors (mEPSC), which are mostly resulted by a single vesicle release. These events are believed to play a role in causing neurobehavioral outcomes such as the withdrawal anxiety (Van Sickle & Tietz, 2002; Xiang & Tietz, 2007) and the hippocampal-dependent learning and memory (Tyler & Pozzo-Miller, 2001). In this context, we studied whether prenatal nicotine exposure could alter basal AMPA-receptor function, which could be evidenced by alterations in mEPSC properties.

The aim of this investigation was to determine the longevity and stability of nicotine's teratogenic effects as well as to establish if AMPA-receptor dysfunction could be one of the neuropathological foundations at the molecular level associated with the observed cognitive deficits.

2. Materials and methods

2.1. Animals, nicotine administration and treatment groups

Female Sprague–Dawley rats ($n = 36$, about 3 months of age) were obtained on day 2 of pregnancy (Zivic-Miller Laboratories, Pittsburgh, PA) and divided into two groups [nicotine group ($n = 23$); saline group ($n = 13$)]. They were implanted on day 3 of pregnancy with 28-day osmotic mini-pumps (Alzet, Cupertino, CA) containing either nicotine bitartrate dissolved in distillate water or physiological saline. Nicotine pumps were filled to deliver 6 mg/kg/day at an average animal weight of 325 g and were located surgically on the back using a short-acting volatile anesthetic (Isoflurane) to provide a subcutaneous route of administration (Murrin et al., 1987). The wound was closed with surgical staples, and the animals were monitored until fully recovered from anesthesia. Body weights of the dams were monitored every third day during pregnancy and lactation. The osmotic mini-pumps were removed under isoflurane anesthesia on the day after delivery.

The day after parturition was considered postnatal day (PND) 1, and litters were culled to an equal number of males and females whenever possible, with an equal number of 10 pups per mother. The progeny was weaned on PND 25, and to control litter effects no more than 1 mate per gender were placed into a particular group (Wainwright, 1998). The colony rooms were maintained at 22–24 °C and kept under a 12 h light/dark cycle. All experiments were performed in accordance with the Principles of Laboratory Animal Care (NIH publication 85-23, 1985) and the protocol approved by Auburn University Institutional Animal Care and Use Committee.

A total number of 200 pups were grouped in six treatment groups [three nicotine (N) and three saline (C)]. The first two groups [one nicotine ($n = 20$/sex) and one saline ($n = 13$/sex) were assessed for developmental milestones, for "anxiety state" in the plus-maze test on PND 40, and for learning and memory on PND 60 using a two-way active avoidance test. A second set of groups using both treatments (nicotine and saline, described above) went through the plus-maze test on PND 60. The other two groups (nicotine and saline) were left undisturbed until the age of six months. They also were examined for anxiety and several days later for cognitive skills.

An additional 10 pups per group were used for electrophysiological analyses at the age of 2–3 months.

2.2. Behavioral and developmental analysis

2.2.1. Developmental analysis

After birth, pups were observed for mortality rates, body weights, incisor eruption and eye opening. Tests for righting reflex and negative geotaxis were performed from the third day of age and continued until all tested animals in two treatment groups met the criteria. Regular observation of the reflex ontogeny is a sensitive indicator for the adaptation of the neonate to extra-uterine life in the earlier stages of the development. Retarded reflexes could be a result of neurological disorders, which might be reflected later in the activity or in the lack of adaptive behavior in the new environment. Complete acquisition of the righting reflex was assumed when the animal could rotate 180° around its longitudinal axis. The animal was placed in the supine position, and the time needed to turn over and to restore normal prone position was recorded (maximum: 2 s). Negative geotaxis was measured by observing the time required for each animal to fully turn and face upwards when placed with its head facing down on 30°-inclined carpeted platform. All control animals achieved the criteria of per-