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Smoking during pregnancy: Postnatal effects on arousal and attentional brain systems

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Abstract

Prenatal exposure to cigarette smoke is known to produce lasting arousal, attentional and cognitive deficits in humans. The pedunculopontine nucleus (PPN), as the cholinergic arm of the reticular activating system (RAS), is known to modulate arousal, waking and REM sleep. Rapid eye movement (REM) sleep decreases between 10 and 30 days postnatally in the rat, with the greatest decrease occurring at 12–21 days. Pregnant dams were exposed to 150 ml of cigarette smoke for 15 min, three times per day, from day E14 until parturition, and the pups allowed to mature. We analyzed (a) intrinsic membrane properties of PPN neurons in slices from pups aged 12–21 days, and (b) the sleep state-dependent P13 auditory evoked potential, which is generated by PPN outputs, in animals allowed to age to adolescence. We found significant changes in the intrinsic membrane properties of PPN cells in prenatally exposed animals compared to intact ones, rendering these cells more excitable. In addition, we found disturbances in the habituation to repetitive stimulation in adolescent, freely moving animals, suggestive of a deficit in the process of sensory gating. These findings could explain some of the differences seen in individuals whose parents smoked during pregnancy, especially in terms of their hypervigilance and increased propensity for attentional deficits and cognitive/behavioral disorders.

Keywords: Arousal; Attention; Nicotine; Pedunculopontine nucleus; Rapid eye movement sleep; Reticular activating system

1. Introduction

Nicotine (NIC) from cigarette smoke can cross into the placenta so that fetuses of mothers who smoke are exposed to higher NIC concentrations in both amniotic fluid and umbilical vein than maternal vein serum (Luck et al., 1985). Coupled with an increased propensity for young women to begin smoking during the childbearing ages of 18–25, prenatal exposure to cigarette smoke represents a critical health problem (Surgeon General, 2002). Toxicological effects of perinatal cigarette smoke exposure include lower birth weight (Eskanazi et al., 1995), higher rate of spontaneous abortion (Kline et al., 1997), and increased incidence of sudden infant death syndrome (SIDS) (Bulterys, 1990). Some effects can have long-term consequences. For example, maternal smoking during pregnancy can lead to increased aggression (Weissman et al., 1999), and problems with sustained attention and impulsivity in adolescent offspring (Fried et al., 1992). Children of smoking mothers are at increased risk for attention-deficit/hyperactivity disorder (Milberger et al., 1997; Wasserman et al., 1999), conduct disorders (Fergusson et al., 1998; Wakschlag et al., 1997) and drug abuse (Weissman et al., 1999), and they are responsible for high rates of violent and persistent criminal offenses (Rasanen et al., 1999; Weissman et al., 1999).

Behavioral deficits generally related to arousal and attentional problems in humans have been identified in rats exposed to NIC prenatally. These animal models show deficits in attention and memory in maze performance (Levin et al., 1993; Sorenson et al., 1991), in learning (Levin et al., 1993), and in operant behaviors (Martin and Becker, 1971). Prenatal NIC exposure is associated with problems with neuronal growth and path-finding (Zheng, 1994), and cell proliferation and differentiation (Lajtha, 1985; Slotkin et al., 1987). Prenatal NIC exposure can induce up-regulation of nicotinic receptors

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(Sershen et al., 1982), to a greater extent on α4b2 subunit containing receptors than on α7 subunit-containing receptors (Van de Kamp and Collins, 1994).

We have been investigating the effects of prenatal exposure to cigarette smoke on brain regions involved in arousal and attention. The pedunculopontine nucleus (PPN), part of the reticular activating system (RAS), is involved in the control of arousal, waking and rapid eye movement (REM) sleep, and disturbances during the development of REM sleep have been proposed to lead to a number of arousal, attentional and behavioral deficits later in life (Garcia-Rill et al., 2003). Fig. 1 shows the major connections of the RAS, especially projections from the PPN to the intralaminar thalamus that are considered to modulate thalamocortical rhythms. We hypothesized that a lack of developmental decrement in REM sleep will lead to lifelong increases in REM sleep drive, such as are present in schizophrenia, anxiety disorders and depression (Garcia-Rill, 1997; Garcia-Rill et al., 2003). Cells in the region of the PPN express α4b2 subunit containing nicotinic receptors (Tribollet et al., 2004). Therefore, we developed a method for exposing pregnant dams to cigarette smoke and studying the effects of such exposure on (a) the intrinsic membrane properties of PPN neurons during the developmental decrease in REM sleep, and (b) the manifestation of the vertex-recorded P13 potential (the rodent equivalent of the P50 midlatency auditory evoked response) which is considered to reflect PPN output in the freely moving animal. These animal models provide an opportunity to characterize cigarette smoke exposure-induced toxicological changes in PPN activity as one of the potential mechanisms underlying the arousal and attentional deficits observed in the offspring of mothers who smoked during pregnancy. The results described herein suggest that major disturbances in the intrinsic membrane properties of PPN neurons, as well as in the manifestation of PPN-generated waveforms in freely moving adolescent animals, could lead to hypervigilance and attentional dysregulation later in life. Preliminary results have been reported in abstract form (Garcia-Rill and Buchanan, 2006).

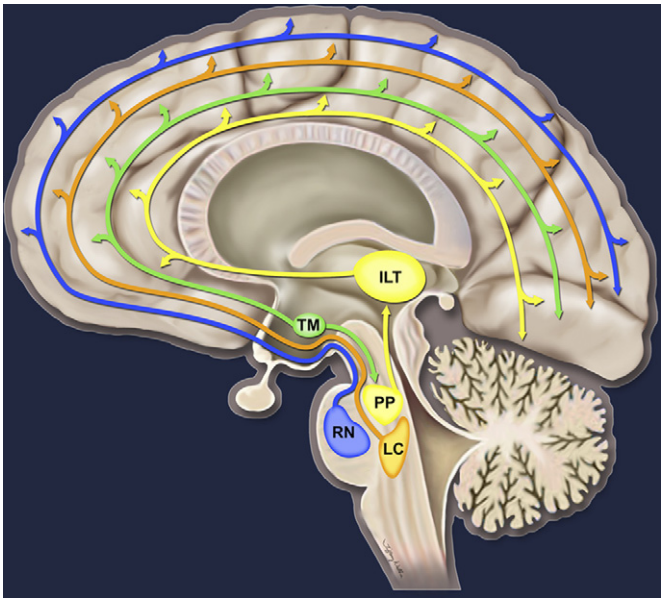

Fig. 1. Ascending connections of the reticular activating system (RAS). The dorsal cholinergic bundle (labeled PP in light yellow for PPN) from the RAS projects to the intralaminar thalamus (ILT) and serves to activate the cortex via thalamocortical projections (also in light yellow). Parallel monoaminergic (noradrenergic locus coeruleus (LC) projections in orange, and serotonergic raphe nuclei (RN) projections in blue) projections travel to the ILT and also directly to the cortex via the ventral bundle. These are joined by ascending projections from the TM (light green for histaminergic tuberomammillary nucleus). In turn, the TM sends descending projections to the RAS that may act reciprocally to stabilize sleep–wake states. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

2. Materials and methods

2.1. Subjects

Pups born to adult timed pregnant Sprague-Dawley rats (Harlan 200–250 g) were used. Pregnant dams were received at embryonic day 10 (E10) and were housed individually in a vivarium with 12:12 light/dark schedule (lights on at 06:00 h) and food and water *ad libitum*. Litters were culled to 10 animals before postnatal day 10. In some litters, when the pups were 12–21 days old, they were anesthetized and rapidly decapitated as previously described (Kobayashi et al., 2003, 2004a,b,c). The brains were dissected free in cooled, oxygenated artificial cerebrospinal fluid (aCSF), and 400 μm slices cut for intracellular recording as previously described (Kobayashi et al., 2003, 2004a,b,c). In other litters, when the pups were 28–30 days old, they were anesthetized and implanted for recording of the P13 potential as previously described (Homma et al., 2003; Mamiya et al., 2005). All animal use procedures were approved by the UAMS and ASU Institutional Animal Care and Use Committees and comply with the ethical standards described in the NIH Guide.

2.2. Smoke exposure

Since PPN neurons are born at E12–14 (Phelps et al., 1990), on day E11, pregnant dams were accommodated to a Plexiglas smoke exposure chamber, and on day 12 exposed once/day, on day 13 twice/day, and on day E14 until parturition, animals were exposed to 150 ml of cigarette smoke from a 1R3F experimental cigarette (Kentucky Tobacco Research and Development Center; 15 mg tar, 1.16 mg nicotine per cigarette) for 15 min three times/day. Smoke was generated by a CH Technologies, model 625A/SG-100 cigarette smoking machine and delivered to the exposure chamber. The amount of NIC in 350 ml of smoke from the 1R3F cigarettes was 204 ± 16 μg (mean ± S.D., $n = 4$). The maximum instantaneous concentration of NIC to which animals were exposed was 24 ± 1.9 ppm. The average exposure over the 15 min exposure period was 9.7 ± 0.8 ppm NIC.

The amount of carbon monoxide (CO) in the chamber was monitored every 2 min using a Monoxor II CO meter (Bacharach Inc., New Kensington, PA). NIC was extracted using anhydrous 2-propanol (Sigma, St. Louis, MO) and stored over a 4 Å molecular sieve to ensure dryness. A Perkin-Elmer