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Brain Research Bulletin 73 (2007) 259–272

BRAIN RESEARCH BULLETIN

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Research report

Lasting effects of nicotine treatment and withdrawal on serotonergic systems and cell signaling in rat brain regions: Separate or sequential exposure during fetal development and adulthood

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Received 21 February 2007; received in revised form 24 March 2007; accepted 25 March 2007
Available online 23 April 2007

Abstract

Neurodevelopmental vulnerability to nicotine extends from fetal stages through adolescence. The recently proposed "sensitization-homeostasis" model postulates that, even in adulthood, nicotine treatment permanently reprograms synaptic activity. We administered nicotine to rats throughout gestation or in adulthood (postnatal days PN90-107), using regimens that reproduce plasma levels in smokers, assessing effects on serotonin (5HT) receptors, the 5HT transporter and responses mediated through adenylyl cyclase (AC). Evaluations were then made on PN105, PN110, PN120 and PN180. Prenatal nicotine exposure elicited persistent suppression of $5HT_{1A}$ receptors and upregulation of $5HT_2$ receptors, effects that were selective for males and that first emerged in young adulthood. In addition, AC activity was reduced and there was uncoupling of receptor-mediated responses. With nicotine exposure restricted to adulthood, there were few changes in 5HT synaptic proteins during treatment or in the first 2 weeks post-treatment, distinctly different from the robust alterations seen earlier with similar nicotine regimens given in adolescence. Nevertheless, there was long-term upregulation of the proteins in males at 6 months of age; females were unaffected. Exposure to prenatal nicotine followed by adult nicotine overcame the protection of females, so that they, too showed long-term effects not seen with either treatment alone; the effects in males were exacerbated in an additive manner. Our results indicate that the effects of nicotine during prenatal or adolescent stages are indeed distinct from the effects in adults, but that even adults show persistent changes after nicotine exposure, commensurate with the sensitization-homeostasis model. These effects may contribute to lifelong vulnerability to readdiction.

Keywords: Adenylyl cyclase; Brain development; Nicotine; Serotonin receptors; Sex differences

1. Introduction

In the developing brain, neurotransmitters such as acetylcholine serve important trophic functions that program the replication, differentiation and phenotypic fate of their target cells [25,26,30]. Accordingly, exogenous agents that interact directly with neurotransmitter receptors are inherently capable of producing lasting disruption of brain development. Nicotine both stimulates and desensitizes nicotinic acetylcholine receptors, thus altering the developmental trajectory of widespread pathways and circuits, culminating in deficits in neural cell numbers, synaptic connections, synaptic function and behavior [31,55,57,58,60]. In this manner, nicotine contributes to the long-term liabilities associated with maternal smoking during pregnancy [19,24,75–77]. It is now evident that the maturation of the brain continues into adolescence and recent work has shown that many of the mechanisms for adverse neurodevelopmental effects of nicotine also operate in the adolescent [59]. In addition, both neurochemical and behavioral evaluations indicate unique response patterns to nicotine administered in adolescence, enhancing the sensitivity to dependence and addiction [1,2,5,12,18,20,59]; the observations in animal models of nicotine administration are echoed by effects seen in adolescent smokers [13–15]. Fetal nicotine exposure also affects the subsequent response to nicotine in adolescence in a manner that reinforces the susceptibility to nicotine dependence [3,4,28,66], likely contributing to the fact that the offspring of women who

Abbreviations: 5HT, 5-hydroxytryptamine, serotonin; AC, adenylyl cyclase; ANOVA, analysis of variance; GD, gestational day; PN, postnatal day

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0361-9230/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2007.03.012

smoke during pregnancy are themselves more likely to become adolescent smokers [28,29,44,47,51].

Given the fact that synaptic rearrangement and even some neurogenic potential persist into adulthood, the question remains as to the extent to which any or all of the fetal or adolescent effects of nicotine are truly "developmental," that is, whether the same types of lasting changes might actually be exerted in adulthood albeit at a lower level of sensitivity. Indeed, it has recently been proposed that nicotine dependence permanently reprograms neural circuitry so that the restoration of normal behavioral function after discontinuing nicotine treatment does not actually connote a return to the pre-exposure state [16]. This hypothesis, the "sensitization-homeostasis" model, predicts that, even in the adult, there are long-term changes in synaptic function that represent the summation of adaptive responses and that leave the brain susceptible to subsequent readdiction, thus accounting for the high likelihood of relapse. We recently explored whether such long-delayed adaptations occur in models of prenatal or adolescent nicotine exposure and found that there are indeed persistent changes in cholinergic and serotonergic (5HT) systems that emerge between adolescence and adulthood [64]. The current study similarly pursues the specific role of 5HT and related cell signaling mechanisms in the issues of persistent or late-emerging effects of nicotine exposure in the prenatal period, adulthood, or the interaction between prenatal nicotine exposure and the response to nicotine administration and withdrawal in the adult.

There are cogent reasons to examine 5HT mechanisms in these models. Tobacco use enhances the likelihood of major depression [23,36,46,78], which is generally thought to reflect abnormalities of 5HT function that contribute both to etiology and therapeutics of depression [35]. Certainly, depressive symptoms are exacerbated during nicotine withdrawal, a significant factor in failed attempts at smoking cessation [11,53,73] and it is clear from animal studies that perturbations of 5HT function play a major role in withdrawal symptoms [48,61,66,70,81]. In our earlier work with adolescent nicotine exposure, we found suppression of 5HT presynaptic activity during withdrawal, in conjunction with alterations in the ability of 5HT receptors to mediate cell signals in their target regions [79,80]; these very same projections are the ones damaged by prenatal nicotine exposure [79]. Accordingly, in the present work, we conducted longitudinal evaluations for up to 6 months of age after either prenatal nicotine exposure or exposure in adulthood. For prenatal nicotine treatment, we focused on effects known to emerge by adolescence [64,66,79] but that undergo a further transition by 6 months of age [64]; here, our interest was to examine the transition between these two stages. For adult nicotine exposure, we concentrated on three aspects: the response of 5HT systems during nicotine exposure, during withdrawal (up to 2 weeks after terminating nicotine treatment) and the subsequent long-term changes at 6 months. In this case, our major interest was to compare the initial and lasting effects of adult nicotine exposure with those seen in adolescent rats [12,59,61,64,66,79] so as to determine whether the adolescent 5HT response pattern is unique. Finally, in our earlier work we detailed how prenatal nicotine exposure alters the subsequent response to nicotine in adolescence, including both the initial effects, changes elicited by withdrawal, and later-emerging alterations at 6 months of age [3,4,28,32,64,66]. Accordingly, we conducted parallel studies of the effect of prenatal nicotine exposure on the response to nicotine in adulthood.

We assessed two 5HT receptor subtypes, $5HT_{1A}$ and $5HT_2$, that are known to be affected in human depression [8,22,82,83] and that are also affected by prenatal or adolescent nicotine administration in rats [59,61,64,66,79,80]. In addition, we evaluated cellular responses mediated through adenylyl cyclase (AC), a signaling pathway that plays critical roles in both dependence and withdrawal [2,43,74], and that we previously found to be affected by withdrawal from adolescent nicotine administration [2,80]. Effects on AC responses can be mediated either through heterologous mechanisms, where changes occur in the level or activity of AC itself, influencing the responses to all receptors that converge on cyclic AMP, or through homologous mechanisms involving changes in the response to a specific receptor. We assessed both alternatives. First, we measured basal AC and maximal activity evoked by a direct stimulant (forskolin) that bypasses neurotransmitter receptors [54]. Second, we determined the AC response to 5HT input. The two 5HT receptor subtypes evaluated here converge on AC through both stimulatory and inhibitory mechanisms [9,17,41,49,52], so we evaluated the net balance of the AC response to 5HT itself, focusing on the shift from stimulatory to inhibitory responses noted previously for adolescent nicotine withdrawal [80]. Determinations were made in the cerebral cortex, which contains major 5HT terminal fields as well as the brainstem, which contains the majority of 5HT cell bodies; for contrast, we also evaluated AC measures in the cerebellum, a region sparse in 5HT, focusing instead on the AC response to β-adrenergic receptor stimulation.

2. Methods

2.1. Animals and nicotine infusions

All studies were carried out with the approval of the Duke University Institutional Animal Care and Use Committee, in accordance with the declaration of Helsinki and with the *Guide for the Care and Use of Laboratory Animals* as adopted and promulgated by the National Institutes of Health. Timed-pregnant Sprague–Dawley rats were shipped on gestational day (GD) 2 by climate-controlled truck (total transit time <1 h), housed individually and allowed free access to food and water. There were four treatment groups: controls (prenatal vehicle + adult vehicle), prenatal nicotine exposure (prenatal nicotine + adult vehicle), adult nicotine exposure (prenatal vehicle + adult nicotine), and those receiving the combined treatment (prenatal nicotine + adult nicotine). On GD4, before implantation of the embryo in the uterine wall, each animal was quickly anesthetized with ether, a 3 cm × 3 cm area on the back was shaved, and an incision made to permit s.c. insertion of type 2ML2 Alzet osmotic minipump. Pumps were prepared with nicotine bitartrate dissolved in bacteriostatic water, to deliver an initial dose rate of 6 mg/kg of nicotine (calculated as free base) per day. The incision was closed with wound clips and the animals were permitted to recover in their home cages. Control animals were implanted with minipumps containing only the water and an equivalent concentration of sodium bitartrate, adjusted to the same pH (3) as the nicotine bitartrate solution. It should be noted that the pump, marketed as a 2-week infusion device, actually takes 17.5 days to be exhausted completely (information supplied by the manufacturer) and thus the nicotine infusion terminates during GD21. Maternal plasma nicotine levels achieved with this administration model resemble those seen in heavy smokers as characterized previously [21,27,31,34,42,56–58,60].