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Increased CRF-like and NPY-like immunoreactivity in adult rats exposed to nicotine during adolescence: Relation to anxiety-like and depressive-like behavior

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Abstract

Objective: Recently, animal models have been developed that demonstrate that adolescent nicotine exposure produces neurobehavioral changes which persist into adulthood. This study further examined the impact of adolescent nicotine exposure on anxiety-like and depressive-like behavior, as well as on levels of corticotropin-releasing factor (CRF) and neuropeptide Y (NPY) in this model.
Methods: Male adolescent rats (35–40 days old) were administered nicotine using Nicoderm CQ™ patches (Smith-Kline Beecham). Behavior in the elevated plus maze (EPM) and forced swim test (FST) was assessed 2–3 weeks after exposure ended. Brain levels of CRF and NPY were then assessed 5–6 weeks after behavioral tests were completed. In addition, blood and brain levels of nicotine resulting from nicotine treatment were examined.
Results: After 5 days of exposure to 5 mg/kg/day nicotine, blood levels of nicotine averaged 66 ± 5 ng/ml and brain nicotine levels averaged 52 ± 4 ng/g. Rats exposed to nicotine displayed an anxiety-like profile in the EPM (i.e., decreased time spent in the open arms) and an antidepressant-like profile in the FST (i.e., less time spent immobile). Rats exposed to nicotine also had increased hypothalamic and frontal cortical CRF, increased hypothalamic and hippocampal NPY, and a decreased ratio of NPY to CRF in the amygdala.
Conclusions: This study demonstrates that adolescent nicotine exposure produces lasting increases in anxiety-like behavior and may reduce depressive-like behavior. These behavioral changes also occurred in concert with alterations in CRF and NPY systems. Thus, lasting neurobehavioral changes associated with adolescent nicotine exposure may be related to allostatic changes in stress peptide systems.

Keywords: Adolescent; Nicotine; Anxiety; Depression; NPY; CRF

1. Introduction

Despite the continued efforts directed at reducing adolescent tobacco use, nearly 20% of adolescents between 12 and 17 years old continue to regularly smoke and use tobacco products (National Household Drug Abuse Survey, 1999). Several studies have provided evidence to suggest that some psychopathologies that occur during childhood and adolescence are associated with tobacco smoking (Breslau et al., 2004). The strongest link between adolescent behavioral disorders and tobacco usage is the presence of conduct disorder (McMahon, 1999). However, stress and anxiety have

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also been strongly associated with smoking behavior in adolescents in both retrospective and prospective studies (Byrnes and Mazanov, 2003). In a recent study adolescent smokers were found to have significantly more anxiety than non-smokers and those with dependence were more anxious than those with no symptoms of dependence on tobacco. The authors further concluded that adolescents with higher measures of trait anxiety were more likely to rely on tobacco "to cope with stress" (Di Franza et al., 2004). It is difficult in retrospective studies measuring stress and anxiety in human adolescents to separate how much pre-existing anxiety influenced the development of smoking as opposed to the contribution of anxiety states that were induced or exacerbated by chronic nicotine exposure and withdrawal. There is some support, however, to suggest that nicotine exposure may increase anxiety in teenagers. For instance in one prospective study of young adults, prior smoking was found to be significantly associated with increased risk for panic attacks/disorder (Isensee et al., 2003). In this light, additional studies demonstrating impaired anxiety or stress-related findings could have important implications regarding human smoking behavior.

Studies in the rodent have proven very useful in assessing differences in sensitivity to nicotine's acute effects in the adolescent relative to the adult (Elliott et al., 2004; Faraday et al., 2001; Levin et al., 2003; Rezvani and Levin, 2004), but also the more persistent consequences of nicotine exposure during adolescence (Faraday et al., 2001; Faraday et al., 2003; O'Dell et al., 2004; Slawecki et al., 2003; Slawecki and Ehlers, 2002; Slotkin, 2002). Animal models also provide data demonstrating that anxiety-like and/or depressive-like behavior is seen in the adult following adolescent nicotine exposure. For instance, rats exposed to nicotine during adolescence are less likely to approach food placed in the center of an open field during a conflict test (Slawecki et al., 2003). This pattern of behavior is indicative of increased anxiety-like behavior (Britton and Thatcher-Britton, 1981). Decreased open field motor activity has also been observed in rats exposed to nicotine during adolescence in some studies (Slawecki et al., 2003; Slawecki and Ehlers, 2002; Trauth et al., 2000c). This hypoactivity could be an index of anhedonia or depressive-like behavior which develops upon cessation of nicotine exposure (Aronen et al., 1996; Markou and Kenny, 2002; Markou et al., 1998). Taken together, these data support the hypothesis that adolescent nicotine exposure could induce anxiety-like and/or depressive-like behavior which persists into adulthood.

Brain corticotropin-releasing factor (CRF) and neuropeptide Y (NPY) are important mediators of anxiety and depression (Heim and Nemeroff, 2001; Redrobe et al., 2002). CRF has been shown to induce, and NPY to suppress, anxiety-like and depressive-like behaviors (Heilig et al., 1989; Spina et al., 1996; Stogner and Holmes, 2000; Stout et al., 2000). Nicotine activates CRF and NPY systems in hypothalamic and extrahypothalamic regions of the brain (Li et al., 2000; Matta et al., 1997). Therefore, it is possible that nicotine exposure during adolescence could produce lasting alterations in CRF and/or NPY systems which could then promote anxiety-like or depressive-like behaviors. A recent study from our laboratory provides some support for this hypothesis. In that study, the effects of CRF on the cortical EEG were found to be attenuated in adult rats that were exposed to nicotine during adolescence (Slawecki and Ehlers, 2003). Since a balance of CRF and NPY may be critical for maintaining behavioral homeostasis (Heilig et al., 1994), further examination of the impact of adolescent nicotine exposure on these neuropeptide systems seems important.

The primary focus of the present study was to examine the impact of adolescent nicotine exposure on anxiety-like and depressive-like behaviors. To accomplish this aim, two behavioral paradigms were used: (1) anxiety-like behavior was assessed using the elevated plus maze (EPM), (2) depressive-like behavior was assessed in the forced swim test (FST). It was hypothesized that exposure to nicotine during adolescence, and its subsequent cessation, would increase anxiety-like behavior and depressive-like behavior because negative affect has been associated with nicotine withdrawal/abstinence (Markou and Kenny, 2002; O'Loughlin et al., 2002). In addition, the effects of nicotine exposure on brain levels of CRF and NPY were assessed in order to determine if alterations in these neuropeptide systems paralleled changes in anxiety-like and/or depressive-like behavior. Based on previous studies (Heilig et al., 1994; Li et al., 2000; Matta et al., 1997), the brain regions examined in the present study included the frontal cortex, hypothalamus, hippocampus, and amygdala. An additional aim of this experiment was to assess the levels of nicotine in blood and the brain produced in this paradigm in order to begin to ascertain the nicotine exposure level sufficient to produce lasting neurobehavioral changes.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats ($n = 51$, 28–30 days old) were obtained from Harlan Sprague–Dawley (Indianapolis, IN). During nicotine treatment, rats were housed 4/cage in standard cages [25 cm (w) × 20 cm (h) × 45 cm (l)]. However, subjects were separated from each other with clear plastic dividers to prevent the removal of the nicotine patch by a cage mate. Rats were pair-housed after exposure ended. A 12-h light/dark cycle (lights on at 6 a.m.) and ad libitum feeding was