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Thyroid receptor β involvement in the effects of acute nicotine on hippocampus-dependent memory

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ABSTRACT

Cigarette smoking is common despite adverse health effects. Nicotine's effects on learning may contribute to addiction by enhancing drug-context associations. Effects of nicotine on learning could be direct or could occur by altering systems that modulate cognition. Because thyroid signaling can alter cognition and nicotine/smoking may change thyroid function, nicotine could affect learning through changes in thyroid signaling. These studies investigate the functional contributions of thyroid receptor (TR) subtypes β and α1 to nicotine-enhanced learning and characterize the effects of acute nicotine and learning on thyroid hormone levels. We conducted a high throughput screen of transcription factor activity to identify novel targets that may contribute to the effects of nicotine on learning. Based on these results, which showed that combined nicotine and learning uniquely acted to increase TR activation, we identified TRs as potential targets of nicotine. Further analyses were conducted to determine the individual and combined effects of nicotine and learning on thyroid hormone levels, but no changes were seen. Next, to determine the role of TRβ and TRα1 in the effects of nicotine on learning, mice lacking the TRβ or TRα1 gene and wildtype littermates were administered acute nicotine prior to fear conditioning. Nicotine enhanced contextual fear conditioning in TRα1 knockout mice and wildtypes from both lines but TRβ knockout mice did not show nicotine-enhanced learning. This finding supports involvement of TRβ signaling in the effect of acute nicotine on hippocampus-dependent memory. Acute nicotine enhances learning and these effects may involve processes regulated by the transcription factor TRβ.

1. Introduction

The development of addiction involves neural and synaptic remodeling, events that also occur during normal learning (Kalivas and O'Brien, 2008). It has been suggested that drugs of abuse, such as nicotine, may usurp the learning and memory machinery at the neural, cellular, and molecular levels to create maladaptive drug-context and drug-cue associations that drive behavior toward addiction (Gould, 2006; Gould and Leach, 2013; Hyman, 2005; Wolf, 2002). Such maladaptive learning may create or strengthen associations between the subjective effects of nicotine and the spatial and discrete cues involved in the drug taking process (i.e., convenience stores, cigarettes, lighters, and packaging) leading to continued drug use (Gould, 2010; Gould and Leach, 2013). Acute nicotine administration in rodents enhanced performance in a variety of hippocampus-dependent learning and memory tasks (French et al., 2006; Gould and Wehner, 1999; Kenney et al., 2012a; Levin and Rose, 1991; Socci et al., 1995) including contextual fear conditioning. Identifying the cell signaling processes underlying the enhancement of learning and memory by nicotine not only advances understanding of learning and memory and addiction but may also identify novel targets for the treatment of nicotine addiction and disorders associated with cognitive decline.

While it has been demonstrated that nicotine enhances contextual memory by acting at nicotinic acetylcholine receptors in the hippocampus (Davis et al., 2007), it is unknown if nicotine is also acting on other systems to modulate learning. One potential target is the thyroid hormone receptor (TR) signaling system. Multiple lines of evidence suggest that nicotine and/or cigarette

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smoking may affect endocrine signaling (For review see Kapoor and Jones, 2005; Tweed et al., 2012) including, but not limited to, thyroid signaling. Specifically, there is a substantial literature describing alterations in thyroid function in current and former smokers (Bertelsen and Hegedus, 1994; Schlienger et al., 2003; Wiersinga, 2013). Smokers generally have higher thyroid hormone levels and lower levels of thyroid stimulating hormone (TSH), which is typically inversely proportional to thyroid hormone level (Christensen et al., 1984; Ericsson and Lindgarde, 1991; Fisher et al., 1997; Jorde and Sundsfjord, 2006); however, there is also evidence that smoking can decrease thyroid function (Soldin et al., 2009). Behavioral and electrophysiological studies in rodents suggest that chronic nicotine can abolish hypothyroidism-induced neural deficiencies. Specifically, chronic nicotine ameliorated learning deficits in rats with experimentally-induced hypothyroidism (Alzoubi et al., 2006b) and nicotine also reversed hypothyroidism-induced deficits in synaptic plasticity (Alzoubi et al., 2006a, 2007). The hypothyroidism-induced deficit in synaptic plasticity was associated with decreases in the activity of learning-related molecules such as CREB and ERK1/2 (Alzoubi and Alkadhi, 2007; Gerges and Alkadhi, 2004). Chronic nicotine ameliorated the molecular, electrophysiological, and behavioral/cognitive disruption caused by induced-hypothyroidism and this may represent convergent or compensatory mechanisms of action. The previously mentioned studies reveal an interaction between the effects of nicotine and thyroid function in experimentally compromised animal models (i.e., thyroidectomized animals that had ~50% normal thyroid hormone levels).

In addition to direct effects of smoking on thyroid function, second hand exposure may also alter thyroid function. Maternal and paternal cigarette smoking may have effects on the thyroid function of their children. The evidence suggests that children of smokers have enlarged thyroids and higher levels of thyroglobulin, a protein that is important in the synthesis of the prohormone thyroxine (T_4), the secreted form of thyroid hormone (Chanoine et al., 1991). Parental smoking is also associated with higher levels of T_4 and lower levels of TSH in infants (Meberg and Marstein, 1986). These studies suggest that chronic exposure to nicotine alters thyroid signaling; however, it is not entirely clear from these studies if these effects are due to nicotine, or due to other components of tobacco smoke, such as thiocyanate (Bertelsen and Hegedus, 1994; Wiersinga, 2013). It is also unclear from human association studies if smoking causes alterations in thyroid hormones or if subjects with alterations in thyroid hormones tend to smoke cigarettes.

The effects of acute nicotine on thyroid function and thyroid hormone receptor (TR) signaling are largely unknown. Animal models suggest that acute nicotine administration did not directly alter thyroid hormone levels (Cam and Bassett, 1983; Huffman et al., 1991), but did reduce TSH levels (Andersson et al., 1988), which is usually indicative of higher thyroid hormone levels. Further, levels of brain thyroid hormones may be independent of serum hormone concentrations, and brain TR activity may not correspond perfectly to serum hormone levels. Thus, this study examined if acute nicotine-associated changes in thyroid signaling are involved in nicotine-enhancement of learning, and if disrupted thyroid signaling alters the effects of acute nicotine on learning. Specifically, this study tested: 1) if nicotine and learning uniquely alter hippocampal transcription factor activity (including thyroid receptor activity) that may relate to its effects on hippocampus-dependent learning, 2) if disrupted TR signaling in knockout mice attenuates the effects of nicotine on hippocampus-dependent learning and memory, and 3) if acute nicotine and contextual fear conditioning have an effect on serum thyroid hormone status.

2. Methods

2.1. Subjects

Subjects used for the transcription factor array experiment (N = 12) and hormone analysis (N = 24) were male C57BL/6J mice (Jackson Laboratories, Bar Harbor ME) 8–12 weeks old at the start of training. Male and female TR mutant mice (TRβ WT and KO (N = 141); TRα1 WT and KO (N = 141)) aged 8–12 weeks at start of training were used for behavioral studies. Mutant mice, originally generated by Forrest, Wikstrom and colleagues, were purchased from Jackson Laboratories. TRβ mutants contained a mutation that disrupted transcription of the entire TRβ gene (TRβ1 and TRβ2) (Forrest et al., 1996b). The TRα1 mutation was specific for TRα1, such that mutant mice expressed functional TRα2 (Wikstrom et al., 1998). Both colonies were backcrossed to parent strain (C57BL/6) for greater than 15 generations, resulting in mice estimated to be >99.9% genetically identical to C57BL/6 mice (Conner, 2002); indicating that they are on a suitable background strain and that results should generalize to the C57BL/6 strain. All mice were maintained in a temperature and humidity controlled vivarium with *ad libitum* access to standard lab chow and water. Mutant mice were bred, maintained, and tested at Temple University according to NIH guidelines. All procedures were approved by the Temple University Institutional Animal Care and Use Committee.

2.2. Apparatus

Fear conditioning training and testing took place in Plexiglas (26.5 × 20.4 × 20.8 cm) conditioning chambers with stainless steel rod grid floors (2 mm diameter) spaced 1 cm apart as previously described (Kenney et al., 2010). Grid floors were connected to a scrambled shock generator (Med-Associates) that delivered 0.57 mA foot shocks. Conditioning chambers, controlled by LabView software, were housed inside sound attenuating chambers (Med-Associates, St. Albans, VT). Each chamber also contained a house light (4 W) as well as a ventilation fan that produced a constant white noise (65 dB) and provided air circulation. Cued fear conditioning testing took place in an altered context. Altered context testing occurred in chambers of a different size (20 × 23 × 19 cm) contained within sound attenuating chambers (Med-Associates, St. Albans, VT) located in a different room from conditioning chambers. The altered context chambers differed in construction in that they had aluminum side-walls and a flat plastic floor. Additionally, vanilla extract was added within each of the chambers to further alter the context. All chambers were cleaned with 70% ethanol before and after each training or testing session.

Auditory startle testing occurred in sound attenuating chambers using SR-Lab Equipment (San Diego Instruments, San Diego, CA). Mice were constrained to Plexiglas cylinders (38 mm internal diameter) that contained a shock grid with 7 rods. The cylinders rested on a platform containing an accelerometer attached to a PC running SR-Lab software.

2.3. Drug preparation and administration

For all experiments, (−) nicotine hydrogen tartrate (reported as freebase weight) was dissolved in physiological saline (Sigma) and all doses were administered at a dose volume of 10 mL/kg. For phenotyping experiments, acute nicotine (0, 0.09, 0.18, or 0.36 mg/kg) was administered via intraperitoneal injection (IP) to mice 5 min prior to the initiation of training and both testing sessions (context and cued). For analysis of serum thyroid hormone levels and the transcription factor array experiment, acute nicotine (0, 0.09, or 0.18 mg/kg) was administered (IP) 5 min prior to contextual fear conditioning training or to a homecage control. Nicotine doses are based on a dose found to produce plasma nicotine levels similar to those of human smokers (Davis et al., 2005).

2.4. Fear conditioning training and testing

For each nicotine dose, TRβ and TRα1 wildtype (WT) and knockout (KO) mice were trained and tested in a combined contextual and cued fear conditioning paradigm (Portugal et al., 2012). Fear conditioning is a useful tool to assess multiple forms of memory and can be conducted in only 2 days, facilitating the examination of acute nicotine's effects on learning. Briefly, mice were placed into conditioning chambers and were allowed to explore for 2 min, at which time a conditioned stimulus (CS, white noise, 85 dB) was presented continuously for 30 s and co-terminated with an unconditioned stimulus (US, footshock) lasting 2 s. After the CS-US pairing, a 2 min inter-trial-interval elapsed prior to a second CS-US pairing. Mice were returned to their homecages 30 s after the second CS-US pairing. 24 h after conditioning, mice were returned to the training context and assessed for freezing for 5 min. Freezing to the training context was used as a measure of hippocampus-dependent contextual memory. At least 1 h after contextual testing, mice were placed into the altered context for 6 min. During the initial 3 min, altered context (pre-CS) freezing was assessed and used as a measure of generalized freezing (Baldi et al., 2004). After the initial 3 min, the CS was presented continuously for an additional 3 min and freezing to the CS was assessed and used to measure hippocampus-independent memory for the CS-US association (Logue et al., 1997; Phillips and LeDoux, 1992).