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The histidine triad nucleotide binding 1 protein is involved in nicotine reward and physical nicotine withdrawal in mice

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HIGHLIGHTS

- HINT1 knockout mice were assessed in nicotine reward and withdrawal models.
- Nicotine reward is absent in HINT1 knockout mice.
- Physical nicotine withdrawal signs were attenuated in HINT1 knockouts.
- Morphine withdrawal signs are present in HINT1 knockout mice.
- The HINT1 gene mediates behaviors relevant to nicotine dependence.

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ABSTRACT

Smoking rates among individuals with schizophrenia are significantly higher than the general population. One possible explanation for this comorbidity is that there are shared genes and biological pathways between smoking and schizophrenia. The histidine triad nucleotide binding protein 1 (HINT1) is a potential candidate, as genetic association and expression studies implicate the gene in both schizophrenia and nicotine dependence; however, the behavioral role of HINT1 in nicotine dependence is unknown. Thus, the goal of the current study was to determine the behavioral role of HINT1 in nicotine dependence. We tested male HINT1 wild-type (+/+) and knockout (−/−) mice in the nicotine conditioned place preference (CPP) test of reward, a nicotine withdrawal model assessing both physical and affective signs, and the nicotine withdrawal conditioned place aversion (CPA) test. HINT1 −/− mice failed to develop a significant nicotine CPP and physical withdrawal signs (hyperalgesia and somatic signs) were attenuated in HINT1 −/− mice. Conversely, HINT1 −/− mice developed a significant nicotine withdrawal CPA similar to their ++ counterparts. Overall, our data support a role for the HINT1 gene in mediating behaviors associated with nicotine reward and physical nicotine withdrawal, and provide insight into the role of HINT1 in nicotine dependence-like behaviors.

1. Introduction

Diseases associated with tobacco use constitute the leading preventable cause of death worldwide [23]. Rates of smoking among individuals with the disabling psychiatric disorder schizophrenia are exceptionally high, with 60–90% of individuals being smokers compared to 20–30% of the general population [4,6,7,13]. While mechanisms underlying schizophrenia and smoking comorbidity are unclear, one hypothesis is that schizophrenia and nicotine dependence share common genes. Recent evidence suggests that the histidine triad nucleotide binding protein 1 (HINT1), a protein expressed in liver, kidney, and mesocortical and mesostriatal brain regions [16] that acts as a tumor suppressor by inhibiting transcription of genes involved in cell proliferation [17], plays a role in both schizophrenia [3,15,19–22] and nicotine-mediated responses.

Specifically, male HINT1 knockout (−/−) mice are less sensitive to the antinociceptive and hypolocomotor effects of acute nicotine responses compared to wild-type (+/+) counterparts, but more sensitive to anxiogenic effects of acute nicotine, indicating a role for HINT1 in acute nicotine behaviors [12]. Human genetic association analysis showed that HINT1 alleles associated with increased risk for schizophrenia are associated with a decreased risk for

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nicotine dependence, as measured by FTND score and the number of cigarettes smoked per day [9]. HINT1 protein levels are also increased after chronic nicotine in the nucleus accumbens (NAc), an effect mediated through nicotinic acetylcholine receptors (nAChRs) [9]. While these genetic and biochemical studies implicate HINT1 in nicotine mediated responses, behavioral studies examining a role for the gene in nicotine dependence are lacking.

In the current study, the goal was to expand on findings from acute behavioral, chronic biochemical, and human genetic studies, and provide the first known study to elucidate the role of HINT1 in important aspects of nicotine dependence in animals. Using male HINT1 ++ and −/− mice, we assessed nicotine reward using the conditioned place preference (CPP) test, and physical (somatic signs and hyperalgesia) and affective (anxiety-related behavior and conditioned place aversion (CPA)) nicotine withdrawal signs. Lastly, because HINT1 was also shown to be involved in morphine-induced behavioral responses [8] we measured somatic signs in HINT1 ++ and −/− mice after morphine withdrawal to determine if the role of HINT1 in nicotine withdrawal extends to other drugs of abuse.

2. Materials and methods

2.1. Animals

Male HINT1 −/− and ++ littermates were used for all studies, generated and bred as previously described [12,17]. Animals were 10–14 weeks of age at the start of experiments and were group-housed in a 21 °C humidity-controlled Association for Assessment and Accreditation of Laboratory Animal Care-approved animal care facility with ad libitum access to food and water. Experiments were performed during the light cycle (8 am–4 pm) and approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. Observations were carried out by an observer blind to the conditions for all studies.

2.2. Drugs

(−)-Nicotine hydrogen tartrate salt and mecamylamine hydrochloride were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). Morphine sulfate and naloxone were obtained from the National Institute on Drug Abuse (Bethesda, MD, USA). Drugs were dissolved in physiological saline (0.9% sodium chloride) and were injected subcutaneously (s.c.) at a volume of 10 ml/kg body weight. All doses are expressed as the free base of the drug.

2.3. Nicotine CPP

Nicotine CPP was conducted over the course of 5 days as described [14]. Briefly, mice (n = 8 per group) were conditioned twice a day for 3 days; once in the morning (7–9 am) and once in the afternoon (2–4 pm), for 20 min with the saline group receiving saline on both sides of the conditioning boxes and drug groups receiving nicotine (0.5 mg/kg) on one side and saline on the opposite side. On test day, locomotor activity counts and time spent on each side were recorded, and data were expressed as time spent on the drug-paired side post-conditioning minus time spent on the drug-paired side pre-conditioning. Positive scores indicated a preference for the drug-paired side, negative scores indicated an aversion to the drug-paired side, and scores at or near zero indicated no preference for either side.

2.4. Nicotine chronic administration

Mice were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) and implanted with Alzet osmotic mini pumps (Durect Corporation, Cupertino, CA) containing (−)-nicotine (36 mg/kg/day) or saline solution for 14 days (withdrawal studies; model 2002) or 28 days (CPA study; model 2004). The nicotine concentration was adjusted according to animal weight and mini pump flow rate.

2.5. Precipitated nicotine withdrawal

Nicotine withdrawal studies were conducted as described [11]. Briefly, on the morning of day 15, saline infused mice (n = 6–8 per group) were injected with saline and nicotine infused mice (n = 8 per group) were injected with the non-selective nAChR antagonist, mecamylamine (2 mg/kg) to precipitate withdrawal signs. Anxiety-like behavior was evaluated for 5 min using the plus maze, followed by a 20-min somatic sign observation including paw and body tremors, head shakes, backing, jumps, curls, and ptosis. Hyperalgesia was evaluated using the hot plate (52 °C) immediately following the somatic sign observation. The number of arm crosses was counted in the plus maze as a measure of locomotor activity.

2.6. Nicotine withdrawal CPA

Nicotine withdrawal CPA was conducted over the course of 4 days as described [10]. Briefly, mice (n = 8–10 per group) were infused with saline or nicotine for 14 days prior to initiation of CPA testing to induce tolerance. Infusion continued throughout the duration of testing. For 2 days, all mice received injections of saline in the morning and were immediately confined to their non-preferred compartment for 30 min. No less than 4 h later, mice received an injection of mecamylamine (3.5 mg/kg) and were immediately confined to their preferred compartment for 30 min. On test day, locomotor activity counts and time spent on each side were recorded. Aversion was counted as mice spending less time in their initially preferred compartment on test day compared to time spent in the same compartment prior to conditioning.

2.7. Morphine withdrawal protocol

Mice (n = 3–5 per group) were injected with saline or morphine over the course of 5 days as follows: Day 1, 50 mg/kg morphine or saline 2× day. Days 2 and 3, 75 mg/kg morphine or saline 2× day. Day 4, 100 mg/kg morphine or saline 2× day. Day 5, 100 mg/kg morphine or saline in the morning. Two hours after the morning morphine or saline injection, mice were injected with vehicle or naloxone (2 mg/kg) to precipitate somatic signs. Somatic signs were observed for 30 min immediately following naloxone or vehicle injection. Somatic signs observed included jumping, paw tremors, head shakes, body tremors, and others (ptosis and diarrhea, scored as one occurrence every 5 min if observed).

2.8. Statistical analysis

Statistical analyses were performed with the Statview® software using two-way analysis of variance with genotype and treatment as between subject factors. p-Values less than 0.05 were considered significant. Significant interactions were further analyzed using the Newman–Keuls post hoc test.

3. Results

3.1. Nicotine does not induce a significant CPP in HINT1 −/− mice

In the nicotine CPP test (Fig. 1), there were significant main effects of treatment ($F_{(1,32)} = 7.27$, $p < 0.05$) and genotype ($F_{(1,32)} = 6.78$, $p < 0.05$) and a significant treatment × genotype interaction ($F_{(1,32)} = 5.33$, $p < 0.05$). Nicotine induced a significant CPP in HINT1 ++ mice; however, failed to produce a CPP response in HINT1 −/− mice. No significant differences in baseline preference