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Research report

Individual phenotype predicts nicotine-haloperidol interaction in catalepsy: Possible implication for the therapeutic efficacy of nicotine in Tourette's syndrome

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HIGHLIGHTS

- The variability in the pro-cataleptic effect of nicotine was investigated in rats.
- Nicotine potentiated haloperidol catalepsy only in rats with low reactivity to stress.
- High reactivity rats were less sensitive to both haloperidol and nicotine.
- The interaction between individual phenotype and drug response is highlighted.
- Results may have implications for the pharmacotherapy of Tourette's syndrome.

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ABSTRACT

In individuals with Tourette's syndrome, the therapeutic efficacy of haloperidol can be augmented by nicotine. In laboratory rats, the dopamine antagonist haloperidol produces catalepsy and nicotine can potentiate it, although this effect is variable and not always observed. Our aim was to understand this variability. In rats, the locomotor response to a novel environment predicts the magnitude of the locomotor response to nicotine. Since the psychostimulant effect of nicotine might counter catalepsy, we hypothesized that rats with a high locomotor response to novelty would show reduced vulnerability to nicotine potentiation of haloperidol catalepsy. First, we administered haloperidol (0, 0.1 or 0.3 mg/kg, ip) and found stronger catalepsy in rats with low reactivity to novelty. Second, we administered haloperidol (0.3 mg/kg) or haloperidol plus nicotine (0.1 mg/kg, ip) and found that nicotine indeed potentiated haloperidol catalepsy but only in rats with low reactivity to novelty. Nicotine did not induce catalepsy on its own. Thus, previously reported inconsistencies in the catalepsy potentiating effect of nicotine may have been due to differential vulnerability to its stimulant actions. As previously observed, the potentiation of haloperidol catalepsy was greatest 4 h after injection. Given the short half-life of nicotine, the mechanism(s) underlying the delayed expression of its pro-cataleptic capacity remains obscure.

1. Introduction

Despite the availability of different drugs for the control of motor abnormalities in Tourette's syndrome, the dopamine antagonist haloperidol continues to be used due to its proven therapeutic efficacy [1]. Nicotine, either in the form of chewing gum or transdermal patch, can potentiate the efficacy of haloperidol in Tourette's syndrome, while having significantly less, if any, therapeutic effect on its own. This finding has been observed in open as well as in double-blind placebo controlled trials [2–6]. The underlying mechanism of the haloperidol–nicotine interaction is currently unknown but potentially important given the adverse short and long-term side effects associated with prolonged exposure to neuroleptics. Some of the more serious neurological side-effects include parkinsonism, dyskinesia, akathisia, dystonia, and potentially the irreversible orofacial movement disorder tardive dyskinesia. Thus, adjunct treatments that could allow a reduction in neuroleptic dose are highly sought [2].

In laboratory rats, haloperidol induces catalepsy, defined as the inability to correct an externally-imposed position. This effect of haloperidol is mediated *via* blockade of striatal dopamine D2 receptors [7]. Interestingly, nicotine can potentiate haloperidol-induced catalepsy while not having any effect on its own [8,9]. This finding is somewhat surprising given that nicotine, a psychostimulant, increases locomotion *via* stimulation of midbrain dopamine cells [10]. Nonetheless, catalepsy may be a useful animal model not

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only from the standpoint of understanding the neurobiology of nicotine–haloperidol interactions in the brain, but also for the possible clinical implications in Tourette's syndrome and perhaps other neuroleptic-responsive disorders as well [11].

In most reports, potentiation of haloperidol catalepsy by nicotine appears to be highly variable, both within and across studies, and has not been uniformly replicated [9,12–15]. In a comprehensive study, we observed a clear enhancement of haloperidol catalepsy in only 3 of 16 experiments, although overall, there was a significant positive trend towards such an effect [15]. The source of this variability is not known, although it has been proposed that a stress–nicotine interaction may be a contributor [14]. Stress has been shown to enhance as well as attenuate different behavioural responses to nicotine in laboratory rats [16–18].

The purpose of this study was to begin to understand the variability associated with the capacity of nicotine to potentiate haloperidol catalepsy. Since nicotine is a psychomotor stimulant, it was reasoned that this property could counter haloperidol-induced catalepsy. Across rats, the stimulatory effect of nicotine and other psychostimulants appears to be a continuum, ranging from weak to strong activational effects. Individual responses to psychostimulant action is positively correlated with the locomotor response to a stressful stimulus, such as the forced exposure to a novel environment [18–21]. We therefore hypothesized that enhancement of haloperidol catalepsy by nicotine would be weaker in rats with high reactivity to novelty.

2. Methods

2.1. Subjects

Male Sprague Dawley rats ($n = 88$) were housed in pairs and allowed to habituate to the animal colony for five days after arrival. Rats were kept in a temperature- and humidity-controlled animal colony lit from 6:30 a.m. to 6:30 p.m. and had unrestricted access to food and water. All testing was carried out between 9 a.m. and 3 p.m. and all experiments were carried out in accordance with the guidelines established by the Canadian Council on Animal Care.

2.2. Reactivity to novelty

On the sixth day after arrival, rats were placed in individual activity chambers and their drug-free locomotor response to the novel environment was counted over a period of 2 h. All rats underwent this test. Each Plexiglas activity chamber was rectangular in shape (in cm: 45 long × 32.5 wide × 37.5 high) and equipped with two parallel infrared photobeams situated 23 cm apart, 11 cm from the short ends of the chambers, and 3 cm above the grid floor. A wire mesh lid above each chamber allowed ventilation. Activity chambers were individually enclosed in sound attenuating cubicles; these were equipped with a fan on the back wall and a 7 W light on the underside of the lid. In-house software registered alternating photobeam interruptions; the total locomotor activity score thus represents the number of times the rat traversed the cage during the test session. Rats were designated as low (LR) or high (HR) responders to novelty based on whether their activity score fell below or above the median of all locomotor activity scores in each experiment. At the end of the test, rats were immediately returned to their home cages. At least three days separated novelty and catalepsy tests. Each rat was used in only one catalepsy experiment.

2.3. Experiment 1: catalepsy response to haloperidol in LR and HR rats

Catalepsy was measured with the bar test and defined as the time required for descent of both paws from the bar (1.2 cm diameter; 14.5 cm long; 10.5 cm above ground). The bar was supported at either end by two parallel plastic walls (21 cm long × 16 cm high) that formed part of a U-shaped unit; the bottom of the unit consisted of a plastic floor (21 cm long × 15 cm wide). In order to reduce visual distractions during catalepsy tests, these portable catalepsy units were placed inside the sound-attenuating cubicles previously used in the novelty test.

Rats ($n = 24$) were weighed and placed into the cubicles, with the catalepsy units already in place, and allowed to habituate to the test environment for 30 min. LR and HR rats ($n = 12$) were then injected subcutaneously with vehicle (0.3% tartaric acid). Catalepsy was measured 1 h later, by lifting the rat by the shoulders and placing its front paws on the bar. The catalepsy score reflects the time taken to remove both paws from the bar. To reduce distractions, rats were mounted onto the bar with their backs to the experimenter and handling was kept to a minimum. At the end of this first test, rats were injected subcutaneously with haloperidol (0.1 mg/kg ($n = 5$ LR, 5 HR) or 0.3 mg/kg ($n = 7$ LR, 7 HR)). The catalepsy response to haloperidol was measured 1 h later. Each rat was tested with only one dose.

2.4. Experiment 2: catalepsy response to haloperidol plus nicotine in LR and HR rats

Following 30 min of habituation in the sound-attenuating cubicles, a second group of rats was injected subcutaneously with haloperidol (0.3 mg/kg: $n = 16$ LR, 16 HR) or vehicle ($n = 16$ LR, 16 HR). The catalepsy response was measured after 1 h. Immediately after this first catalepsy test, half of the rats in each group received an intraperitoneal injection of nicotine (0.1 mg/kg, free base) or vehicle (0.9% saline). Catalepsy was again determined at 1 and 4 h post-injection. To minimize handling and sensorimotor stimulation due to changing environments, rats remained in their respective cubicles for the entire duration of the test (5.5 h). Drug doses in Experiments 1 and 2 were chosen from within the framework of previous studies showing that 0.1 mg/kg nicotine can potentiate haloperidol catalepsy (0.2–0.4 mg/kg) [8,12,13,15].

2.5. Drugs

Haloperidol (Research Biochemicals Inc., Natick, MA) was dissolved in 0.3% tartaric acid in distilled water and the pH was adjusted to 5.5 with NaOH. *S*(−)-Nicotine ditartrate (Sigma Chemical Co., St. Louis, MO) was dissolved in 0.9% saline and the pH adjusted to 7.2 with NaOH. Haloperidol and nicotine were kept frozen (−80 °C) until just prior to use and were injected in a volume of 1 ml/kg.

2.6. Data analysis

Statistical analysis was carried out using commercial software: Statistica 6.0 (Stat-Soft, Tulsa, OK). In Experiment 1, total catalepsy scores obtained with each dose of haloperidol were subjected to a two-way mixed ANOVA with phenotype (LR, HR) and treatment (vehicle, haloperidol) as between and within factors, respectively. In Experiment 2, baseline catalepsy scores obtained with haloperidol alone were analyzed with a *t*-test for independent samples. Haloperidol plus nicotine scores were analyzed with a three-way mixed ANOVA (haloperidol × nicotine × time), with time as the repeated measure. For both experiments, *post hoc* comparisons between individual means were carried out with Tukey's honestly significant difference (HSD) tests. The correlation between the locomotor response to novelty (2 h total) and the catalepsy potentiating effect of nicotine was evaluated with Pearson's correlation coefficient.

3. Results

Two-way ANOVAs revealed that haloperidol at 0.1 and 0.3 mg/kg produced catalepsy only in LR rats (Fig. 1a and b). At the 0.1 mg/kg dose, we observed a significant effect of treatment ($F(1, 8) = 16.16$, $p < 0.005$) and a significant treatment × phenotype interaction ($F(1, 8) = 6.32$, $p < 0.05$), with only LR rats showing significant catalepsy ($p < 0.01$ *vs* vehicle, Tukey HSD test). Analysis of variance on the scores obtained with the 0.3 mg/kg dose revealed a significant effect of treatment ($F(1, 12) = 20.92$, $p < 0.001$), phenotype ($F(1, 12) = 7.11$, $p < 0.05$) and an interaction ($F(1, 12) = 5.54$, $p < 0.05$). Tukey *post hoc* analysis indicated that haloperidol produced significant catalepsy in LR rats ($p < 0.005$) and that the response to haloperidol was stronger in LR *vs* HR rats ($p < 0.05$).

In Experiment 2, only the 0.3 mg/kg dose of haloperidol was used, based on previous studies demonstrating nicotine potentiation of haloperidol catalepsy [8,13,15]. Comparison of mean haloperidol catalepsy scores with *t*-tests for independent samples revealed significant catalepsy in both LR ($t(30) = 6.05$, $p < 0.01$) and HR ($t(30) = 4.18$, $p < 0.01$) rats (Fig. 2a and c). Again, the magnitude of the catalepsy response in LR rats was significantly greater than that observed in HR rats (348.8 ± 51.70 s *vs* 168.6 ± 35.06 s; $t(30) = 2.89$, $p < 0.01$).

In LR rats, the three-way ANOVA revealed a significant interaction ($F(1, 28) = 11.70$, $p < 0.005$) and *post hoc* Tukey analysis showed that nicotine potentiated haloperidol-induced catalepsy 4 h after injection ($p < 0.05$; Fig. 2b). Nicotine alone did not produce catalepsy nor did it increase haloperidol catalepsy 1 h after injection. In HR rats, nicotine did not enhance haloperidol-induced catalepsy at either time point (interaction: $F(1, 28) = 0.26$, $p = 0.61$; Fig. 2d).

Analysis of the relation between total locomotor response to a novel environment and potentiation of haloperidol catalepsy by