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Agonist and antagonist effects of cytisine *in vivo*

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ABSTRACT

Varenicline, the most successful smoking cessation aid, is a selective partial agonists at α4β2* nicotinic receptors. Its efficacy is likely to be shared by other drugs with similar receptor action, including cytisine. The present study aimed to characterize behavioral effects of cytisine compared with nicotine using locomotor activity tests, intracranial self-stimulation of ventral tegmental area (discrete-trial threshold current intensity titration procedure), drug discrimination (0.6 mg/kg nicotine from vehicle), physical dependence (osmotic minipumps delivering 6 mg/kg/day of nicotine) and intravenous nicotine self-administration (0.01 mg/kg per infusion) in adult Wistar rats. Cytisine (1–3 mg/kg) partially substituted for nicotine and at the highest dose tended to antagonize nicotine's discriminative stimulus effects. Nicotine (0.05–0.4 mg/kg), but not cytisine (0.3–3 mg/kg), lowered ICSS thresholds and cytisine dose-dependently reversed effects of nicotine. Nicotine (0.15–0.6 mg/kg), but not cytisine (0.3–3 mg/kg), stimulated locomotor activity and cytisine (3 mg/kg) fully reversed these effects of nicotine. Acute pretreatment with nicotine (0.15–0.6 mg/kg), but not cytisine (0.3–3 mg/kg), reinstated extinguished nicotine self-administration. Continuous infusion of nicotine induced physical dependence, as indicated by reduced rates of food-reinforced responding induced by a challenge dose of mecamylamine. At the highest tested dose (3 mg/kg), cytisine tended to reduce response rates irrespective of whether the rats were continuously exposed to nicotine or saline. Cytisine behaves like a weak partial agonist, mimicking effects of nicotine to a limited degree. Although cytisine reversed several effects of nicotine, it seemed to have a reduced potential to produce withdrawal signs in nicotine-dependent subjects.

1. Introduction

Stimulation of neuronal nicotinic receptors is currently the most effective treatment strategy for tobacco smoking cessation. Varenicline, the most successful drug in this class, is a fairly selective partial agonist of α4β2* nicotinic receptors. Varenicline is clearly effective in the clinic, resulting in significantly higher quit rates and higher continuous abstinence rates *vs* placebo (Nides et al., 2006; Oncken et al., 2006).

Being a partial agonist, varenicline is expected to antagonize effects of nicotine where the patient smokes while alleviating a withdrawal state while the patient abstains from smoking (Rollema et al., 2007b). Preclinical data seem to support these expectations. On one hand, varenicline fully substituted for nicotine in drug discrimination studies (LeSage et al., 2009; Rollema et al., 2007a), lowered brain stimulation reward thresholds (Spiller et al., 2009), and enhanced locomotor activity (Zaniewska et al., 2008). On the other hand, varenicline blocked nicotine-induced dopamine release, reduced nicotine self-administration in rats and supported lower self-administration break point than nicotine (Rollema et al., 2007a).

For several decades preceding the introduction of varenicline into clinical practice, there was another nicotinic receptor ligand, cytisine, in clinical use in Eastern and Central European countries as a smoking cessation agent (Etter et al., 2008). Like varenicline, cytisine is a partial agonist acting on α4β2* nicotinic receptors. It was argued though that cytisine has a limited blood–brain barrier penetration and this limits its efficacy (Rollema et al., 2010). Nevertheless, recent clinical analysis suggested that cytisine does facilitate smoking cessation and may be an affordable alternative to other more expensive treatment options (Hajek et al., 2013; West et al., 2011; Walker et al., 2014). Although effect sizes in cytisine trials may be similar to those observed in varenicline trials, a

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head-to-head comparison of varenicline and cytisine is necessary before any conclusions on cytisine's noninferiority can be drawn (Walker et al., 2014).

The goal of the present study was to characterize interactions between nicotine and cytisine using several behavioral paradigms. Firstly, a drug discrimination study aimed to guide cytisine dose selection for subsequent experiments. It was previously reported, similar to varenicline albeit with ca. 10-fold lower potency (Rollema et al., 2010), cytisine is able to substitute for nicotine. Present experiments were conducted with a range of doses of cytisine spanning from the dose that produced no nicotine-like discriminative stimulus effects up to the dose that produced maximum substitution for nicotine. Secondly, drug discrimination studies were paralleled by experiments on locomotor activity and intracranial self-stimulation (ICSS) where effects of nicotine and cytisine were again compared. In ICSS studies, drugs like nicotine reduce stimulation thresholds determined using rate-independent methods and, at low doses levels, varenicline was reported to produce nicotine-like facilitation of self-stimulation behavior (Spiller et al., 2009). Thirdly, while nicotinic receptor antagonists such as mecamylamine are known to precipitate withdrawal signs in subjects with nicotine physical dependence, partial agonists may be less likely to do so. Nicotine dependence is often studied by observing subtle behavioral symptoms associated with the withdrawal state (Malin et al., 1992). An alternative and potentially more sensitive approach is based on detecting withdrawal-induced interruption of food-maintained operant behavior and was successfully applied to studies for both spontaneous and antagonist-precipitated withdrawal in nicotine-dependent rats (Vann et al., 2006). Finally, as the acute presentation of nicotine is known to reinstate nicotine seeking behavior (Shaham et al., 1997), the ability of cytisine to prime extinguished nicotine self-administration behavior was assessed.

Results of the present studies confirm the findings reported recently by another group (Igari et al., 2014) that cytisine behaves *in vivo* like a weak partial agonist. Cytisine was found to possess weak nicotine-like discriminative stimulus effects but failed to stimulate locomotor activity, to facilitate intracranial self-stimulation or to reinstate nicotine seeking behavior. Although a higher dose of cytisine readily antagonized several effects of nicotine, it seemed to have a reduced potential to produce withdrawal signs in nicotine-dependent subjects.

2. Experimental procedures

2.1. Subjects

Adult male (nicotine discrimination, self-administration, locomotor activity and behavioral dependence) and ovariectomized female (intracranial self-stimulation) Wistar rats (State Breeding Farm "Rappolovo", St. Petersburg, Russia) weighing 250–300 g (12–13 weeks of age) at the beginning of the experiments were used. Animals were housed individually in standard rodent cages (31 cm × 21 cm × 15 cm, length × width × height; Velaz, Czech Republic) or in groups (N = 5; only for locomotor activity experiments) in standard rodent cages (55 cm × 32 cm × 18 cm, length × width × height; Velaz, Czech Republic) with wood-chip bedding (Tier-Wohl, JRS J. Rettenmaier & Söhne GmbH+Co. KG, Germany). Filtered tap water (filter AQUAPHOR®, St. Petersburg, Russia) and standard rat lab chow (Laboratorsnab, Moscow, Russia) were available *ad libitum* throughout the experiments (locomotor activity and self-stimulation) or food consumption was restricted to 14–16 g/day given after behavioral testing to limit the body weight gain to 5–6 g/week (nicotine discrimination, self-administration and behavioral dependence).

Artificial light was provided daily from 08.00 to 20.00 and room temperature and humidity were at 21 ± 1 °C and 40–70%, respectively. Experimental procedures were approved by the Ethics Committee of Pavlov State Medical University and were performed in accordance with the recommendations and policies of the U.S. National Institutes of Health "Guide to the care and use of laboratory animals".

2.2. Drugs

(−)-Nicotine hydrogen tartrate (Sigma–Aldrich Co., St. Louis, MO, USA) and cytisine (Sequoia Research Product Ltd, Pangbourne, UK) were dissolved in phosphate buffered saline (pH = 7.4). Mecamylamine (Sigma–Aldrich Co., St. Louis, MO, USA) was dissolved in saline. Solutions of all drugs were made fresh daily and administered in a 1 ml/kg injection volume. Nicotine doses refer to the weight of the salt except for self-administration and behavioral dependence experiments. In the self-administration experiment, nicotine was self-administered at a dose of 0.01 mg/kg per infusion (free base). In the behavioral dependence experiment, ALZET osmotic pumps (model 2ML2, Alza Corporation, Palo Alto, CA, USA) were loaded with nicotine dissolved in saline and were expected to deliver 6 mg/kg nicotine (free base) in a volume equivalent to 0.12 ml/day.

2.3. Apparatus

Except for locomotor activity, all experiments were conducted in standard operant conditioning chambers (RITEC, St. Petersburg, Russia) enclosed within sound-attenuating ventilated cubicles. Chambers were equipped with two nose-poke operanda (RITEC, St. Petersburg, Russia; self-stimulation and self-administration experiments) or response levers (MED Associates Inc., East Fairfield, VT, USA; nicotine discrimination and behavioral dependence experiments), food delivery mechanisms (MED Associates, Inc., East Fairfield, VT) delivered 45-mg food pellets (MLab Rodent Tablet, TestDiet®, Richmond, IN, USA), nicotine delivery lines and pumps (RITEC, St. Petersburg, Russia) and other input/output devices (MED Associates, Inc., East Fairfield, VT) described below. Chambers were connected to an operating PC through MED interface (MED Associates Inc., East Fairfield, VT, USA). In intracranial self-stimulation experiments, electrical pulses were produced by constant current stimulators (PHB-150B; MED Associates Inc., East Fairfield, VT, USA). The electrical stimuli were delivered to the animal through a two-channel electrical swivel assembly (Plastic One, Roanoke, VA, USA), which extended into the test chamber. The electrical stimulus was a 500 ms train of rectangular bipolar waves with pulse frequency of 100 Hz and pulse duration of 0.1 ms. Throughout the experiments the electrical stimuli were displayed on the oscilloscopes (C1-55, Russia) which permitted to determine whether or not the stimulator was functioning properly.

In locomotor activity experiments, motor activity was measured in two sets of five identical boxes each (25 × 35.5 × 34 cm) with transparent Plexiglas walls and a non-transparent plastic floor enclosed within sound-attenuating ventilated cubicles. Light intensity inside the apparatus was 30–40 Lx. Boxes were equipped with three infrared photocell beams (5 cm off the floor) for measuring horizontal activity and eight infrared photocell beams (14 cm off the floor) for measuring vertical component of motor activity connected to an operating computer through an interface and controlled by MED-PC software.

2.4. Experiment 1: nicotine discrimination

Rats were initially shaped to lever press for food pellet delivery according to a fixed-ratio 1 (FR1) schedule of reinforcement using the lever, which would eventually become the 'vehicle' lever. All training and subsequent acquisition sessions were conducted once daily six days a week. After rats acquired the lever-press response (1–3 days), the FR value was gradually increased to 10. The active lever was then switched to the opposite side and the FR value was reduced to FR1. As soon as the rats showed evidence of responding on this lever ('nicotine' lever), the FR value was rapidly increased to FR10.

At the start of each drug discrimination training session, rats were injected subcutaneously (s.c.) with either 0.6 mg/kg of nicotine or vehicle, returned to their home cages, and then 5 min later were placed into the operant conditioning chambers for 15 min. The house light was illuminated at the start and extinguished at the end of the session. Animals were subjected to both 'vehicle' (V) and 'nicotine' (N) training sessions in an alternating sequence predetermined for each two-month block of training and testing: (1) VNVNVVN, NVVVNVV, NNNVNVN, VNNVVNV and (2) NVNVNNV, VNNNVNN, VVVNVNV, NVVNNVN. During the training sessions, both levers were present in the chamber but only correct lever pressing was reinforced under the FR10 schedule of pellet delivery. Half of the rats were trained to press the right lever after receiving nicotine injections and the left lever following vehicle injection; the reverse pairing was used with the remaining rats. Incorrect responses reset the FR requirement on the correct lever.

Acquisition training proceeded until the following criteria were met in ten consecutive training sessions: 1) the first ten consecutive responses had to occur on the correct lever; 2) the percentage of all lever presses emitted on the correct lever was more than 90% during these sessions. After the criteria were met, rats were given test days. During the 15-min test sessions, ten consecutive responses on either lever produced a pellet delivery. Tests were conducted provided that the following criteria were met: 1) during the most recent training sessions of each type ('vehicle' or 'nicotine') the first ten consecutive responses occurred on the correct lever; 2) overall 90% or greater correct-lever responding on each of these sessions; 3) overall response rate was greater than 0.2 lever presses per second. Each rat was repeatedly tested with either vehicle or the training dose of nicotine (0.6 mg/kg) until four completed consecutive test sessions satisfied the criteria described above.

Substitution (stimulus generalization) and antagonism tests were conducted with cytisine (1–3 mg/kg, s.c.; 15 min pre-session injection time) or its vehicle. Cytisine was given in combination with either nicotine (0.075–0.6 mg/kg, s.c.; 5 min