Neuropharmacology 105 (2016) 463–470

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Varenicline impairs extinction and enhances reinstatement across repeated cycles of nicotine self-administration in rats

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ARTICLE INFO

Article history:
Received 20 November 2015
Received in revised form
11 February 2016
Accepted 15 February 2016
Available online 18 February 2016

Keywords:
Nicotine
Varenicline
Rat
Self-administration
Extinction
Reinstatement

ABSTRACT

Varenicline is a partial nicotine receptor agonist widely prescribed as a smoking cessation medication. Repeated (or long-term) use of varenicline has been proposed as a treatment option for tobacco addiction. However the effect of repeated varenicline use on motivation for nicotine is unknown. Here the intravenous nicotine self-administration paradigm in rats was used to model the consequences of varenicline treatment across repeated cycles of administration, extinction and reinstatement. Rats acquired nicotine self-administration across 20 days before undergoing 6 days of extinction, where each extinction session was preceded by a single injection of varenicline or saline. This was followed by a single varenicline-free nicotine-primed reinstatement test. All rats then reacquired nicotine self-administration for 10 days followed by a second cycle of extinction. Across this period, rats either received a second cycle of varenicline (VAR–VAR) or saline (SAL–SAL), or the alternative treatment (SAL-VAR, VAR-SAL), followed by a final reinstatement test. Treatment with varenicline increased responding across the first cycle of extinction, but did not affect responding in the reinstatement test. Across the second cycle, varenicline again increased responding across extinction, and critically, rats treated with varenicline across cycle 1 and saline across cycle 2 (Group VAR-SAL) exhibited more reinstatement than rats in any other group. The effect of VAR on nicotine seeking was not due to its effects on locomotor activity. Instead, the results suggest that a history of VAR can increase vulnerability to reinstatement/relapse when its treatment is discontinued. The possible mechanisms of this increased vulnerability are discussed.

1. Introduction

Tobacco smoking is highly addictive and has severe negative health consequences. It is responsible for the death of one in 10 adults worldwide (World Health Organisation, 2008), with recent estimates indicating that 60% of smokers will die of a smoking-related illness (Banks et al., 2015). Unfortunately, less than 2% of smokers successfully quit each year (Giovino, 2002) with an average of 12–14 attempts required before any lasting abstinence is achieved (Partos et al., 2013; Zhu et al., 2007). To improve the chances of quitting at any one attempt, many smokers resort to the use of popular anti-smoking treatments or medications. The most successful medication currently available for the treatment of tobacco dependence is the partial nicotine receptor agonist varenicline (Cahill et al., 2013). Through competitive binding to the nicotinic acetylcholine receptor (nAChR), VAR blocks the subjective rewarding effects of nicotine, and at the same time produces a moderate increase in mesolimbic dopamine release to alleviate cravings and withdrawal (Coe et al., 2005; Niaura et al., 2006).

Clinical research into the effectiveness of VAR treatment has largely focused on outcomes following a single quit attempt (Cahill et al., 2013). Evidence that repeated use of NRT and bupropion may improve the likelihood of achieving long-term abstinence is unclear: Ellerbeck et al. (2009) reported that the probability of quitting may increase across attempts, Cupertino et al. (2009) found no significant gains and Tonnesen et al. (1993) suggest that repeated use of NRT may be efficacious, but only if the initial quit attempt was pharmacotherapy-free. It is presently unknown whether VAR treatment across repeated quit attempts increases the likelihood of achieving long-term abstinence.

This question is difficult to address in people due to issues associated with the reliability of self-report measures, and the

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http://dx.doi.org/10.1016/j.neuropharm.2016.02.023
0028-3908/

influence of other factors, including a smoker's expectations about the effects of VAR and the use of concurrent counselling (Cupertino et al., 2009; Ellerbeck et al., 2009; Tonnesen et al., 1993). For this reason, preclinical models of nicotine use may provide a useful means of assessing the impact of repeated cycles of VAR on the inhibition of nicotine seeking. One such model is extinction and reinstatement of nicotine intravenous self-administration in rats. Using this model, VAR has been shown to dose-dependently reduce nicotine self-administration (George et al., 2011; Le Foll et al., 2012; O'Connor et al., 2010) and nicotine-primed reinstatement (O'Connor et al., 2010), with the impact on cue-induced reinstatement less clear (Le Foll et al., 2012; O'Connor et al., 2010; Wouda et al., 2011). Surprisingly, these models have yet to address the impact of VAR when administered across extinction of nicotine-seeking, the exact period when a smoker is most likely to use anti-smoking medication to facilitate a quit attempt.

There are at least two reasons why the use of VAR across a period of extinction may predispose rats to relapse/reinstatement. First, any learned control over nicotine seeking in extinction may be encoded with respect to the internal state induced by VAR. If so, then removal of this state upon cessation of VAR treatment would effectively renew extinguished nicotine seeking responses. Second, independently of any state dependent effects, VAR may reduce rats' drug seeking across extinction, and therefore, the amount of inhibitory learning that is required to oppose the tendency towards nicotine seeking. Hence, when exposed to a cue (external or internal) that may precipitate nicotine-seeking, there is less inhibitory control present to prevent relapse.

Accordingly, the aim of the current study was to assess the impact of chronic VAR treatment on the rate at which nicotine seeking is extinguished, and thereafter, the magnitude of its reinstatement. We additionally examined the effects of VAR across an additional cycle of self-administration, extinction and reinstatement. It was anticipated that VAR would substitute for nicotine and prime responding across extinction, resulting in higher levels of responding across this phase, and by the reasoning outlined above, that treatment with VAR would increase the magnitude of reinstatement when the treatment ceased. An initial control experiment assessed whether the selected dose of VAR produced locomotor effects that could interfere with the self-administration protocol.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats (175–200 g; Animal Resource Centre, Perth, Australia) were housed 4 per cage on a 12 h reverse dark/light cycle (lights on 1900h). Food was initially available *ad libitum* and then restricted to 22 g/rat/day following recovery from surgery. All procedures were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (8th edition, 2013) and were approved by the Animal Ethics Committee of the University of New South Wales.

2.2. Drugs

(−)-Nicotine hydrogen tartrate (Sigma–Aldrich, St Louis, MO, USA) and varenicline tartrate (7, 8, 9, 10 − Tetrahydro-6, 10 methano-6-H-pyrazino[2, 3-h][3]benzazepine tartrate; Tocris Bioscience, Bristol, United Kingdom) were dissolved in sterile sodium chloride solution (0.9%). Nicotine and varenicline doses refer to the base and salt respectively.

2.3. Apparatus

The experiments were conducted in sixteen standard self-administration chambers (Med Associates, VT; 30.5 × 24 × 29 cm) consisting of clear plexiglass panels (front, back, and ceiling), aluminium panels (left and right) and a stainless steel rod floor (19 rods, 10 mm apart, 4 mm diameter) above a tray filled with corn cob bedding. To measure locomotor activity, four infrared photobeams were located 600 mm apart and 15 mm above the metal rods on the front and back panels. Chambers were housed in individual sound attenuation boxes fitted with ventilation fans.

Each chamber contained two nose-pokes on the right aluminium panel, spaced 14 cm apart and 1.5 cm above the grid floor. Each nose poke contained a single LED. Syringe pumps were located outside the sound attenuation boxes and were connected via tubing to a weighted fluid swivel assembly (Instech, Pennsylvania, USA). This was attached to a spring connector which was connected to the backmount of each rat. Data for all experiments were recorded on a Windows XP PC by MED-PC IV software.

2.4. Procedure

2.4.1. Experiment 1: the effect of varenicline on locomotor activity

This experiment examined the time course of the locomotor response to a single injection of VAR. The aim was to identify the dose and time-point at which any activity dissipates. This will confirm that any changes in activity detected across self-administration are not due to the treatment with VAR. Locomotor testing was carried out in self-administration boxes with nose-poke holes blocked and tethers removed.

Rats (n = 8) were familiarized with the activity chambers across two consecutive days for 120 min each day. This was done to achieve a low baseline of activity prior to injection. On the second of these days, rats were removed from the chambers after 30 min and administered a single subcutaneous (s.c.) injection of sterile saline (0.9%, 1 ml/kg) before returning to the test chamber for a further 90 min.

The following four test sessions followed the same format as day 2, with the exception that the rats received injections of 0, 0.3, 1 or 3 mg/kg varenicline (Goutier et al., 2015; Igari et al., 2014) in a counterbalanced order across tests according to a Latin square design. The tests were separated by two rest days to allow for varenicline to washout between test sessions (Obach et al., 2006).

Locomotor activity was recorded at all times and the house light was on for the duration of all familiarization and test sessions.

2.4.2. Experiment 2: effect of varenicline on repeated cycles of nicotine self-administration, extinction, and reinstatement

2.4.2.1. Surgery. One week after arrival, rats (n = 48) were implanted with a chronic indwelling catheter into the right jugular vein. Briefly, rats were anaesthetised with 2–3% inhalation isofluorane in oxygen (2 L/min) and injected with a pre-emptive analgesic (Carprofen, 5 mg/kg s.c.). A custom made silastic catheter was then inserted into the jugular vein and terminated in the heart. The distal end passed subcutaneously to exit posterior to the scapulae and terminated with a 22 gauge back mount cannula (Plastics One, VA, US). The back mount was secured in place with suture and flushed daily with cephazolin sodium antibiotic (0.2 ml, 100 mg/ml) in sterile saline (0.9%) and heparin (150 I.U/ml). Rats were allowed seven days to recover from surgery before commencement of testing. At the end of all experimental procedures catheter patency was verified by a single IV infusion of ketamine (0.1 mL, 10 mg/kg). A patent catheter was evident as instant loss of muscle tone.