T-Type Calcium Channel Antagonism Decreases Motivation for Nicotine and Blocks Nicotine- and Cue-Induced Reinstatement for a Response Previously Reinforced with Nicotine

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Background: Recent evidence suggests an involvement of T-type calcium channels in the effects of drugs of abuse.

Methods: We examined the influence of the novel, potent, and selective T-type calcium channel antagonist [2-(4-Cyclopropylphenyl)-N-((1R)-1-{5-[2,2,2-trifluoroethyl]oxo}pyridine-2-yl)ethyl]acetamide] (TTA-A2) (.3, 1, or 3 mg/kg) on motivation for nicotine, as measured by nicotine self-administration on a progressive ratio (PR) schedule, and nicotine- and cue-induced reinstatement for a response previously reinforced with nicotine delivery ($n = 11$ or 12 Long Evans rats/group). Furthermore, we examined the specificity of the TTA-A2 effects by characterizing its influence on PR responding for food (in the absence or presence of nicotine-potentiated responding), food- versus nicotine-induced cue-potentiated reinstatement for a response previously reinforced by food administration ($n = 11$ or 12 Wistar Hannover rats/group), and its ability to induce a conditioned place aversion.

Results: TTA-A2 dose-dependently decreased self-administration of nicotine on a PR schedule and the ability of both nicotine and a cue paired with nicotine to reinstate responding. The effects were specific for nicotine's incentive motivational properties, as TTA-A2 did not influence responding for food on a PR schedule but did attenuate the ability of nicotine to potentiate responding for food. Likewise, TTA-A2 did not alter food-induced cue-potentiated reinstatement for a response previously reinforced by food but did decrease nicotine-induced cue-potentiated reinstatement. Finally, TTA-A2 did not produce an aversive state, as indicated by a lack of ability to induce conditioned place aversion.

Conclusions: These data suggest that T-type calcium channel antagonists have potential for alleviating nicotine addiction by selectively decreasing the incentive motivational properties of nicotine.

Key Words: Addiction, drug abuse, incentive salience, progressive ratio, relapse, self-administration

T-type calcium channels are characterized by activation at low voltages, rapid inactivation, slow deactivation, and small single channel conductance (see [1,2] for reviews). Because of their involvement in burst firing (3,4) and pacemaker activity (5,6), these channels have been hypothesized to be involved in disorders hallmarked by alterations in electroencephalogram signaling (1). However, as selective antagonists for this channel have not been identified until recently (7,8), the hypothesis that T-type calcium channel antagonism might have therapeutic utility for central nervous system disorders has remained untested (9).

In addition to a potential role in sleep and seizure liability (4,10), T-type calcium channels might also be involved in the effects of drugs of abuse. For example, Newton *et al.* (11) showed that the mixed N-type and T-type calcium channel antagonist NP078585 reduced the reinforcing effects of alcohol, although these effects were attributed to the compound's N-type channel activity. Biala and Budzynska (12) have found that the nonselective calcium channel antagonists nimodipine and flunarizine block nicotine-induced conditioned place preference. In a more recent publication, Urbano *et al.* (13) reported that injections of cocaine given in a "binge-like" fashion elevated T-type current amplitudes and reduced the threshold for activating thalamic neurons. They proposed that such changes could lead to the thalamocortical dysfunctions observed in human cocaine abusers (14–16). Finally, the marginally selective T-type calcium channel antagonists divalent nickel and sFTX-3.3 reduced nicotine-induced calcium mobilization in substantia nigra pars compacta neurons (17), an effect that would be predicted to decrease nicotine-induced dopamine release in vivo. This is important because of the involvement of dopamine signaling in the abuse potential of nicotine and other addictive drugs (18–20).

Given this evidence, we were interested in examining the influence of a selective T-type calcium channel antagonist on the reinforcing and incentive motivational effects of nicotine. We have recently identified a potent, brain penetrant, state-dependent, selective antagonist, [2-(4-Cyclopropylphenyl)-N-((1R)-1-{5-[2,2,2-trifluoroethyl]oxo}pyridine-2-yl)ethyl]acetamide] (TTA-A2) (8,21,22). We tested the ability of TTA-A2 to influence nicotine self-administration on a progressive ratio (PR) schedule and nicotine-induced and cue-induced reinstatement for a response previously followed by nicotine administration. To characterize the selectivity of the observed effects and rule out potential confounds, we took advantage of the fact that nicotine increases the incentive motivational properties of nondrug rewards by acting as a so-called reinfocement enhancer, an

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attribute thought to contribute to nicotine's addictive potential (23). We compared the effect of TTA-A2 on 1) PR responding for a food reward in the absence and presence of nicotine-induced reinforcer enhancement and 2) food-induced versus nicotine-induced cue-potentiated reinstatement for a response previously resulting in a food reward. We predicted that if TTA-A2 selectively decreased the incentive motivational properties of nicotine, it would attenuate nicotine-induced potentiated PR responding and reinstatement but not responding for food alone or food-induced reinstatement. On the other hand, if TTA-A2 reduced motivation in general (or produced some confounding effect on responding), an effect on responding for food reward alone was expected.

Methods and Materials

Experiment 1: Influence of TTA-A2 on Responding for Nicotine on a PR Schedule and Nicotine and Cue-Induced Reinstatement

Animals. Male Long Evans rats (250–275 g) purchased from Charles River Laboratories (Wilmington, Massachusetts) were housed singly in a humidity- and temperature-regulated vivarium on a 12/12 hour reverse light/dark cycle. Water and chow were available ad libitum until training and subsequently rats were fed 25 g chow per day following their daily operant session.

Apparatus. Nicotine self-administration occurred in operant chambers controlled by a computer interface system (Med Associates, St. Albans, Vermont). Chambers were equipped with two levers 2.5 cm above a grid floor. Active lever presses activated a microliter syringe pump. Inactive lever presses were recorded but had no consequences. A cue light was positioned 7.5 cm above the active lever and a tone generator was located directly above the cue light. A house light was located on the opposite side of the chamber and signaled the onset of the session.

Food Training and Surgery. Before surgery, rats underwent operant training for 45-mg sucrose pellets on a fixed ratio 1 (FR1) reinforcement schedule. Rats were then anesthetized and prepared with catheters implanted into the right jugular vein as previously described (24,25); see Supplement 1 for more information.

Effect of TTA-A2 on Responding for Nicotine Under a PR Schedule. Rats were trained to self-administer nicotine (.03 mg/kg/infusion) on an FR1 schedule for five daily 1-hour sessions. Each nicotine infusion was followed by a 40-second time-out in which the conditioned stimulus (CS+) light was delivered but lever presses had no consequence. Rats were then placed under fixed ratio 2 and fixed ratio 5 schedules for two and six sessions, respectively. Animals were then trained for 4 days to respond for nicotine on a PR schedule until stable performance was achieved (Table 1), as has been previously reported (26–28). The sequence of PR schedule was determined using the exponential formula $5e^{(.2 \times [\text{infusion number} + 3])} - 5$, with the first three values replaced by 3, 6, and 10. The PR sessions concluded following 20 minutes of inactivity on the active lever.

Table 1. Nicotine Infusions Earned During Progressive Ratio Training

Day of PR Training	Infusions ± SE
1	8.91 ± .39
2	9.87 ± .4
3	10.2 ± .46
4	10.69 ± .51

PR, progressive ratio.

Once animals reached stable PR performance, the effects of TTA-A2 on responding were examined using a between-subjects group design (n = 11–12 per group). Animals were administered either vehicle or TTA-A2 (.3, 1, or 3 mg/kg orally) 1 hour before testing. The highest dose tested in this and the following studies (3 mg/kg) was chosen based on Uebele *et al.* (8), indicating that it is the minimum effective dose for producing changes in electroencephalogram activity, a marker of target engagement. In addition, these doses are not expected to have off-target activity, because administration of 10 mg/kg TTA-A2 produces cerebrospinal fluid concentrations (which are thought to reflect free brain levels) of ~100 nmol/L, yet TTA-A2 has no significant activities in >170 functional and binding assays when tested at 5 μmol/L or less (8).

Effect of TTA-A2 on Cue- and Priming-Induced Reinstatement. Following PR testing, all rats were retrained on a fixed ratio 5 schedule (1-hour session) for 3 days. Extinction of nicotine self-administration was then carried out, during which responding on the active lever did not result in a nicotine infusion. For half of the animals, the tone and cue light were still presented following an active lever press (priming group), whereas the tone and cue light were absent (cue group) for the other animals. Rats were given extinction sessions (10–24 sessions) until they achieved extinction criteria of less than 15 lever presses on the active lever on consecutive sessions.

Cue-induced reinstatement of nicotine seeking was examined in rats that had undergone extinction in the absence of tone and light cues. The cue-induced reinstatement sessions were essentially identical to the extinction sessions for these animals except that active lever presses resulted in the delivery of the light and tone cue that were previously paired with nicotine delivery. The effects of TTA-A2 (.3, 1, and 3 mg/kg orally) on cue-induced reinstatement were evaluated in a Latin square design. Extinction sessions (minimum of one session) were conducted between test days.

Reinstatement of nicotine seeking induced by nicotine priming (.15 mg/kg subcutaneous) was examined in rats having undergone extinction in the presence of tone and light cues. Reinstatement sessions were essentially identical to the extinction sessions except that nicotine was injected 15 minutes before the session. The effects of TTA-A2 (.3, 1, or 3 mg/kg orally) on reinstatement induced by priming were examined using a Latin square design.

Experiment 2: Influence of TTA-A2 on PR Responding for a Food Reward in the Absence or Presence of Nicotine-Induced Potentiation and Nicotine- and Food-Induced Cue-Potentiated Reinstatement

Animals. Adult male Wistar Hannover rats (Charles River) weighing 200 g to 250 g at the time of arrival were individually housed under a 12-hour light/dark cycle. Animals were acclimated to the colony room for 7 days with food and water available ad libitum and then food restricted (16 g food per day) before training and the rest of the experiment. All animal procedures were conducted under Institutional Animal Care and Use Committee guidelines.

Apparatus. Sixteen standard rat operant boxes (Med Associates) were employed. Each box contained two retractable levers with a stimulus light located above each lever. A food hopper in which 45 mg sucrose pellets (Bioserv, Frenchtown, New Jersey) could be delivered was centered between the left and right levers.

Training. Animals were first trained to respond for food on an FR1 schedule as described previously (29). Rats were then