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Decreasing nicotinic receptor activity and the spatial learning impairment caused by the NMDA glutamate antagonist dizocilpine in rats

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ABSTRACT

Nicotinic systems have been shown by a variety of studies to be involved in cognitive function. Nicotinic receptors have an inherent property to become desensitized after activation. The relative role of nicotinic receptor activation vs. net receptor inactivation by desensitization in the cognitive effects of nicotinic drugs remains to be fully understood. In these studies, we tested the effects of the α7 nicotinic receptor antagonist methyllycaconitine (MLA), the α4β2 nicotinic receptor antagonist dihydro-β-erythroidine (DHβE), the nonspecific nicotinic channel blocker mecamylamine and the α4β2 nicotinic receptor desensitizing agent sazetidine-A on learning in a repeated acquisition test. Adult female Sprague–Dawley rats were trained on a repeated acquisition learning procedure in an 8-arm radial maze. MLA (1–4 mg/kg), DHβE (1–4 mg/kg), mecamylamine (0.125–0.5 mg/kg) or sazetidine-A (1 and 3 mg/kg) were administered in four different studies either alone or together with the NMDA glutamate antagonist dizocilpine (0.05 and 0.10 mg/kg). MLA significantly counteracted the learning impairment caused by dizocilpine. The overall choice accuracy impairment caused by dizocilpine was significantly attenuated by co-administration of DHβE. Low doses of the non-specific nicotinic antagonist mecamylamine also reduced dizocilpine-induced repeated acquisition impairment. Sazetidine-A reversed the accuracy impairment caused by dizocilpine. These studies provide evidence that a net decrease in nicotinic receptor activity can improve learning by attenuating learning impairment induced by NMDA glutamate blockade. This adds to evidence in cognitive tests that nicotinic antagonists can improve cognitive function. Further research characterizing the efficacy and mechanisms underlying nicotinic antagonist and desensitization induced cognitive improvement is warranted.

1. Introduction

Nicotinic acetylcholine receptors have been shown by a variety of studies to be critically involved in cognitive function (for review see (Levin et al., 2006)). These receptors are targets for cognitive enhancement research to help with diseases like Alzheimer's disease, attention deficit hyperactivity disorder, and schizophrenia (Levin, 2002; Wallace et al., 2011). The critical actions of nicotinic agonists at nicotinic receptors for these effects are still not well understood.

It is important to note that an inherent property of nicotinic receptors is to become desensitized after activation (Ochoa et al., 1989). The relative role of nicotinic receptor activation vs. net inactivation by desensitization for cognitive enhancing as well as other functional effects of nicotinic agonists remains to be fully understood, but nicotinic receptor desensitization may provide therapeutic effects including cognitive improvement (Buccafusco et al., 2009; Levin et al., 2013; Picciotto et al., 2008) and nicotinic antagonists may also have therapeutic benefits (Dwoskin and Crroks, 2001).

Though high doses of nicotinic antagonists have been shown to impair memory (Levin et al., 1987), modestly decreased nicotinic receptor activation by receptor desensitization or blockade can improve cognition. Low doses of the nonspecific nicotinic antagonist mecamylamine had memory enhancing effects in rats and monkeys (Terry et al., 1999). Chronic infusions of mecamylamine improved working memory in the radial-arm maze (Levin et al., 1993). Relevant to the current tests of learning, we showed that low-dose acute administration of mecamylamine significantly

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reduced repeated acquisition errors (Levin and Caldwell, 2006). In a clinical study, low dose mecamylamine was found to improve recognition memory in adults with ADHD (Potter et al., 2009). These studies suggest that some cognitive improvement seen with nicotine and other agonists may be the result of receptor desensitization following activation, rather than the activation itself.

Previous studies have shown that attention can be improved through nicotinic receptor desensitization. Acute administration of the α4β2 nicotinic receptor desensitizing agent and partial agonist sazetidine-A improved attentional performance on an operant visual signal detection task, reversing the attentional impairments caused by either the NMDA glutamate antagonist dizocilpine or the muscarinic acetylcholine antagonist scopolamine (Rezvani et al., 2011). Chronic sazetidine-A infusions were also found to improve attentional performance on the same task and to significantly attenuate scopolamine-induced attentional impairment (Rezvani et al., 2012). To determine whether the sazetidine-A effects resulted from its desensitizing effect or from its partial agonist effect at α4β2 nicotinic receptors, we tested the effect of the α4β2 nicotinic receptor antagonist DHβE on the same task. Acute DHβE attenuated attentional impairment caused by dizocilpine (Levin et al., 2013). On the same task, the α7 nicotinic antagonist MLA also showed efficacy in reversing dizocilpine-induced attentional impairment. This finding is in line with previous research into the effect of low dose MLA on attentional enhancement (Hahn et al., 2011).

This further exploration of the efficacy of modestly decreasing nicotinic receptors for cognitive improvement was conducted to provide better understanding of the complex nature of nicotinic receptor involvement with cognitive function and to explore new avenues for development of nicotinic therapies for cognitive dysfunction.

2. Materials and methods

2.1. Subjects

Young adult female Sprague–Dawley rats were used in the current set of studies ($N=11–12$/study). Female rats were selected for use in these studies to facilitate comparisons of the current results with previous results with nicotinic antagonist effects on other cognitive tasks like the attentional signal detection task. For an entire series of studies over 20 years of testing of nicotinic drug effects on cognitive function we have used female rats because they maintain relatively constant body weight throughout adulthood. Thus alterations in pharmacokinetics would not be a factor in drug effects on behavior. The rats were tested in a repeated measures counterbalanced design with the treatments given at multiple time points which would have been scattered throughout the estrus cycle so that estrus phase would not confound the drug effects. Separate sets of rats were used to test each of the three nicotinic antagonists. The rats were housed in groups of 2–3 with freely available water and feedings made each day to keep the subjects at approximately 85% of unrestricted feeding body weight adjusted for growth to provide motivation for the appetitively motivated repeated acquisition test. These studies were conducted under approval of the Duke University Institutional Animal Care and Use Committee.

2.2. Drug treatments

In four different experiments the effects of the α7 nicotinic receptor antagonist methyllycaconitine (MLA), the α4β2 antagonist dihydro-β-erythroidine (DHβE), the nonspecific nicotinic antagonist mecamylamine and the nicotinic α4β2 desensitizing agent sazetidine-A (Georgetown University, Washington, DC, USA for sazetidine-A and Sigma, St. Louis, MO, USA for the other drugs) were tested for their effects in reversing the impairments caused by the NMDA antagonist dizocilpine on learning in a repeated acquisition test. Adult female Sprague–Dawley rats were trained on a repeated acquisition learning procedure in an 8-arm radial maze. Each day each rat was presented with a different array of three arms, which were rewarded with a food pellet for the first entry. The other five arms were not reinforced. The rats were tested for five trials per day to determine their learning of the new daily problem. Training continued until the rats reliably showed a learning curve when each daily new problem was presented. This took approximately 21 training sessions. Then three experiments were conducted in separate sets of rats in a repeated measures counter-balanced design with different dose sequences for each rat, a range of MLA doses (0, 1, 2 and 4 mg/kg), DHβE doses (0, 1, 2 and 4 mg/kg), mecamylamine doses (0, 0.125, 0.25 and 0.5 mg/kg) or sazetidine-A (0, 1 and 3 mg/kg) were administered either alone or together with the NMDA glutamate antagonist dizocilpine (0, 0.05 or 0.10 mg/kg) s.c. 20 min before the beginning of the test. The doses chosen were those that we previously found to effectively attenuate dizocilpine-induced impairment of accuracy on the attentional task. (Levin et al., 2013). The drug doses were given a repeated measures counterbalanced design.

The drugs used like all others have complex actions. DHβE shows preference for blocking α4β2 nicotinic receptors, but also does some effects at α7 receptors (Papke et al., 2008). MLA is a competitive antagonist that has been found to have selectivity for α7 vs. α4β2 nicotinic receptors (Marks et al., 1999), but there is evidence that it also has activity at α4β2 nicotinic receptors as well (Karadsheh et al., 2004). Mecamylamine is a noncompetitive nicotinic channel blocker without much selectivity among nicotinic receptor subtypes (Papke et al., 2008). Sazetidine-A is a mixed agonist and desensitizing agent at α4β2 nicotinic receptors (Xiao et al., 2006; Zwart et al., 2008)and recently has been found to have some actions at α7 nicotinic receptors (Brown and Wonnacott, 2014).

An automated radial-arm maze (Med Associates Inc., Georgia, VT, USA) was used. The rats were trained on an automated 8-arm radial maze elevated 5 cm from the floor with a central platform of 30 cm in diameter and walls 32.5 cm height from which extend the arms with the dimensions of $17.5 \times 12.5 \times 67.5$ cm^3. Clear Plexiglas walls are at the sides and on the top of each arm. Each arm is separated from the central platform by vertical aluminum gates. Feeders are located at the end of each arm and feed one pallet (P.J. Noyes Co Inc.) at a time. The maze was in a room that contained extra-maze visual cues. The cues were always kept in the same position when testing. The rats were first handled for 5 min for a few days to accustom them to human contact. They were then shaped by being placed in the center of the maze with 15 pellets and kept there until all the pieces had been eaten or a maximum of 15 min had ended. Once the rats had consumed the food reinforcers within the 15 min allocated, training on the maze was started. This involved baiting 3 of the 8 arms with reinforcers. The same 3 arms were kept baited for an individual rat for 5 continuous trials in which they chose arms until they had selected the three baited arms or a maximum of 3 min elapsed. Then the next trial was immediately started with the return of the rat to the center of the maze, and after 10 s the doors to the arms were opened. Different random combinations of arms were baited in different sessions. Not more than two adjacent arms would be baited. To start the session, the rat was placed in the central cylinder and the program would start after 10 s. The gates open allowing rats free movement around the maze for 3 min or until all baited arms were chosen. To be considered an entry, the rat had to enter the arm and walk to the end. Entries to any arms other than the first time entry to the baited arms were counted as errors.

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