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Ketanserin attenuates nicotine-induced working memory improvement in rats

Edward Levin *, Laura Icenogle, Ashkan Farzad

Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, United States

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Abstract

Nicotinic systems have been shown in numerous studies to be important for spatial working memory. Nicotinic systems are certainly not acting alone in the basis of memory function, but act in concert with a variety of other neural systems. Important for these interactions is nicotinic induced release of a variety of neurotransmitters involved in memory function including serotonin (5-HT). We have found that the 5-HT_2 receptor antagonist, ketanserin, effectively attenuated nicotine-induced attentional improvement. The current study explored the interaction between nicotinic and serotonergic systems in the performance of a spatial working memory task in the radial-arm maze. Female Sprague–Dawley rats were trained on the win-shift working memory task on the 8-arm radial maze. After 18 sessions of acquisition training the rats were given acute doses of nicotine (0.2 and 0.4 mg/kg), ketanserin (0.5, 1 and 2 mg/kg) or combinations of the two. The vehicle served as the control. As seen in previous studies, nicotine caused a significant improvement in working memory performance as indexed by the number of correct arm entries before the first error (entries to repeat). Ketanserin at the doses tested did not cause a significant effect on choice accuracy, but it did significantly attenuate the improvement caused by the 0.2 mg/kg nicotine dose. The higher 0.4 mg/kg nicotine dose was nearly sufficient to overcome the ketanserin effect. This study shows that, as with attentional function, nicotine-induced working memory improvement is attenuated by the 5-HT_2 antagonist ketanserin. Given that many antipsychotic drugs have substantial 5-HT_2 antagonist effects, these atypical antipsychotic drugs may reduce the cognitive improvements caused by nicotinic treatments.

Keywords: Nicotine; Ketanserin; Working memory; Radial-arm maze

1. Introduction

Nicotine and nicotinic agonists can significantly improve attention and working memory (Addy and Levin, 2002; Levin and Rezvani, 2000; Levin and Simon, 1998; Rezvani and Levin, 2001). Nicotinic therapies hold promise as novel treatments for cognitive disorders, including schizophrenia, attention deficit hyperactivity disorder (ADHD) and Alzheimer's disease (Newhouse et al., 1997; White and Levin, 1999). The mechanisms of nicotinic treatment with regard to interactions with other neural systems involved in memory function need to be elucidated to help in the development of novel nicotine treatments for cognitive dysfunction.

Nicotine can interact with a variety of transmitter systems by virtue of its action stimulating the release of numerous neurotransmitters, including acetylcholine, dopamine, norepinephrine, serotonin, and glutamate (Wonnacott et al., 1989). These cascading effects of nicotine can be important with regard to its interactions with other drugs. Antagonists of the receptors of neurotransmitters released by nicotine can attenuate nicotine effects. This would demonstrate which streams of the nicotinic cascade are important for the functional effects of nicotine. Also, in a practical sense, nicotinic interactions with drugs affecting other receptor systems can be important for potential co-therapeutic use of nicotine and those drugs.

Nicotine use is particularly high in people with some types of psychopathology. People with schizophrenia smoke at very high rates, with 88% of adults in a schizophrenic population being smokers (Hughes et al., 1986), more than a three times greater rate of smoking than the general population. Smokers with schizophrenia may be self-medicating with nicotine to improve their cognitive function (Adler et al., 1993; Kelly and McCreadie, 2000; Levin et al., 1996). Smoking improves auditory sensory gating in schizophrenics (Adler et al., 1993).

* Corresponding author. Psychiatry and Behavioral Sciences, Box 3412, Duke University Medical Center, Durham, NC 27710, USA. Tel.: +1 919 681 6273; fax: +1 919 681 3416.
E-mail address: edlevin@duke.edu (E. Levin).

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Nicotinic co-treatment may be useful for improving cognitive function in patients with schizophrenia. Nicotinic drugs are being developed for possible use as co-treatments for the cognitive impairment of schizophrenia. However, interactions of nicotinic drugs with antipsychotic drugs may influence their effectiveness.

In the case of schizophrenia treatments, a classic mechanism of action of antipsychotic drugs such as haloperidol is dopamine D_2 blockade (Matsubara et al., 1993; Sumiyoshi et al., 1993). Dopaminergic D_2 blockade has been shown to attenuate nicotine-induced memory improvement (Addy and Levin, 2002). Atypical antipsychotic drugs such as clozapine have additional actions including pronounced blockade of serotonergic 5-HT_2 receptors, which is considered to add to their therapeutic antipsychotic effects (Matsubara et al., 1993). Serotonergic 5-HT_2 blockade can also affect nicotinic actions on cognitive function. Ketanserin, much like clozapine, is a 5-HT_2 receptor antagonist (Ruotsalainen et al., 1997). With regard to attentional function, we recently found that the 5-HT_2 antagonist ketanserin significantly diminished nicotine-induced improvement in selective attention (Rezvani et al., 2005). The current study was conducted subsequently in a separate set of rats to determine if 5-HT_2 blockade with ketanserin would also block nicotine induced working memory improvement in the radial-arm maze.

2. Methods

2.1. Subjects

Adult female albino Sprague–Dawley rats ($N=11$) were used in this experiment. The rats which were not used for any other study were kept three per cage, with one cage containing a rat that did not learn the maze and thus did not participate in the study. The cages were maintained in a room which operated on a reverse 12-h light:dark cycle, beginning with the dark cycle at 7:00 AM. One week before the study began, the rats were on an ad lib rodent chow diet and were also provided four pieces of Kellogg's Froot Loops® cereal for each day. Once the study began, the rats were fed daily after testing a diet of rodent chow that gradually increased throughout course of the study, but to keep them at a healthy lean weight. The behavioral testing was conducted during the dark cycle and the rats were immediately fed the rodent chow pellets after testing. All procedures were conducted in accordance with established standards and policies of animal treatment by the Duke University Animal Care and Use Committee.

2.2. 8-arm radial maze

The working memory was tested on a black-painted, eight-arm radial maze constructed of wood. The height of the maze above the floor was 30 cm and the central platform, from where the eight arms protrude radially outward, had a diameter of 35 cm. Each arm was equally distanced from one another and each had a width of 10 cm and a length of 80 cm. Before the training acquisition and drug trials were started, two shaping sessions were conducted by placing a 30 cm black plastic ring on the central platform and a rat inside of it with 8 half pieces of Froot Loops cereal. The rat remained in the ring until it had consumed all 8 pieces of Froot Loops, or until 300 s had elapsed.

During testing sessions, the maze was thoroughly wiped down with a paper towel, the eight arms were baited with a half piece of sweetened cereal (Froot Loops, ®Kellogg's, Battle Creek, MI, USA) in a food cup at each end, and the black plastic ring was placed on the central platform. A rat was placed inside the ring for 10 s and then the ring was removed to allow the rat to run the maze. The rat ran the maze for 300 s, or until all 8 arms were entered. An entry occurred when all four legs of the rat had crossed the threshold of the arm (i.e. the boundary between the central platform and the opening of an arm). The choice accuracy was recorded as the entries to repeat (ETR), the number of arm entries made before an arm was repeated. The total time of the trial was recorded and the latency was determined by dividing this time by the total number of arm entries the rat had made. All the trials on the radial-arm maze were performed in the same room, with sufficient visual cues placed in the room in plain view of the rat during each trial.

2.3. Ketanserin–nicotine interactions

A total of 18 training sessions were completed on the radial arm maze. Then, two practice sessions, using a volume of 1 ml/kg saline were subcutaneously injected into the rats to habituate them to the injection process. After these two days of saline injections to habituate the rat to the injection procedure, testing began for the acute administration of nicotine and ketanserin. The doses of ketanserin were 0, 0.5, 1, and 2 mg/kg, the doses of nicotine ditartrate were 0, 0.2, and 0.4 mg/kg, and they were co-administered via subcutaneous injections in a volume of 1 ml/kg, 20 min (sc) prior to the start of each trial on the radial arm maze. The doses of the drugs were calculated on the basis of the salt weights. Each dose of the drugs was dissolved in a solution of 85% saline and 15% dimethylsulfoxide (DMSO). The vehicle was a solution of 85% saline and 15% DMSO, which was used for the control injections. Each rat was tested with each of the drug combinations on the radial-arm mazes, with all the drug treatments being counterbalanced with at least two days between doses. Then, the entire group of drug doses was repeated a second time.

3. Results

Before the drug challenge began, the rats completed 18 training sessions on the radial-arm maze. They had reached asymptotic levels for their choice accuracy scores of entries to repeat (ETR) before the end of the 18-session acquisition training phase.

Nicotine administered by itself significantly improved the working memory performance at both the 0.2 mg/kg ($p<0.05$) and the 0.4 mg/kg ($p<0.05$) doses, compared to saline (Fig. 1). This nicotine cognitive effect has been seen in many earlier

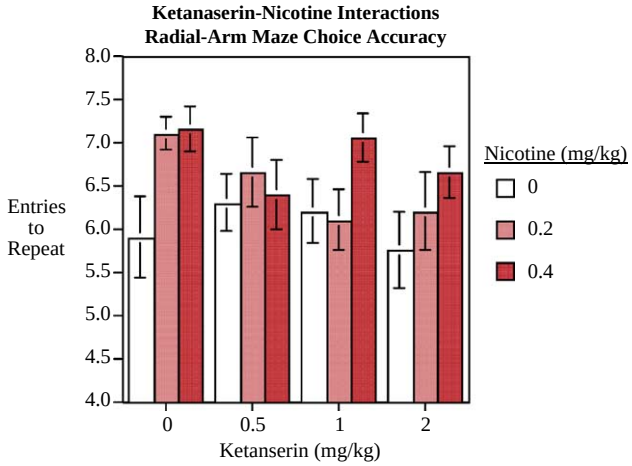

Fig. 1. Ketanserin interactions with nicotine and radial-arm maze working memory performance, entries to repeat (mean ± SEM).

experiments (Addy and Levin, 2002; Levin and Simon, 1998). Ketanserin in the dose range tested (0.5–2 mg/kg) did not significantly affect working memory accuracy when it was given alone. Co-administration of ketanserin with nicotine was seen to diminish the memory-improving effect of nicotine. Neither of the nicotine doses significantly improved choice accuracy when given together with ketanserin. The effectiveness of the 0.2 mg/kg nicotine dose was completely suppressed by ketanserin. There was a trend of the higher 0.4 mg/kg nicotine dose overcoming the suppression by ketanserin at the 1 and 2 mg/kg doses, but this was not quite significant ($p < 0.06$ at 1 mg/kg ketanserin and $p < 0.09$ at 2 mg/kg ketanserin).

Response latency showed somewhat different effects. As shown in Fig. 2, ketanserin (2 mg/kg), when administered alone, significantly ($p < 0.01$) increased latency compared with control. Nicotine (0.4 mg/kg), when administered alone, significantly ($p < 0.025$) reduced the latency as compared to the saline control. The reduction in latency at 0.4 mg/kg of nicotine was significantly attenuated by 0.5 mg/kg of ketanserin ($p < 0.05$). However, this was not seen with the higher ketanserin doses.

4. Discussion

Acute nicotine (0.2–0.4 mg/kg) improved the working memory performance of rats on the radial-arm maze in the current experiment, replicating the same effect seen in a variety of previous studies (Levin and Simon, 1998). Acute ketanserin at the 0.5–2.0 mg/kg dose range did not significantly affect working memory performance. This is similar to the findings of Ruotsalainen et al. who evaluated the effects of this dose range of ketanserin on working memory function. (Ruotsalainen et al., 1997). When given in combination, ketanserin significantly attenuated the memory improvement caused by 0.2 mg/kg of nicotine. This blockade of nicotine-induced memory improvement may have been absolute inasmuch as the higher dose of 0.4 mg/kg of nicotine showed a trend toward memory improvement even when administered with 1 or 2 mg/kg of ketanserin.

The current finding that ketanserin by itself did not significantly affect working memory is similar to the finding of Ruotsalainen et al., who demonstrated that ketanserin in the same dose range, along with two other 5-HT antagonists, methysergide and methiothepin, did not impair working memory performance on the delayed non-matching to position task, nor did ketanserin significantly reduce the attentional performance on the 5-choice serial reaction time task (Ruotsalainen et al., 1997). As in their study, ketanserin seemed to increase the latency of the rats in this study. Recently, we also found in the 16-arm radial maze that the same dose range as used in the current study did not impair choice accuracy in a more difficult task (Aldridge et al., 2005). Ketanserin was not without effect inasmuch as rats that had developmental exposure to the organophosphate pesticide chlorpyrifos showed a dose-related ketanserin induced choice accuracy impairment.

The behavioral effects underlying ketanserin interactions with nicotine effects on radial-arm maze choice likely involve effects on working memory function. However, it is possible that ketanserin interactions with nicotine on attention may underlie the effects. Previously, we found that like in the current study in the radial-arm maze, ketanserin attenuated nicotine-induced improvement in attentional function as measured on an operant signal detection task (Rezvani et al., 2005). One problem with the hypothesis that attentional effects are at the basis of the ketanserin-nicotine interactions on radial-arm maze choice accuracy is that the effective dose range for nicotine improving performance on the operant attention task is an order of magnitude lower than the effective dose improving choice accuracy in the radial-arm maze.

The neural mechanisms of ketanserin interactions with nicotine likely involve its blockade of $5HT_{2A}$ receptors. Ketanserin is a potent antagonist at $5HT_{2A}$ receptors. It less potently blocks $5HT_{2c}$ receptors, and has little appreciable effect on $5HT_1$, $5HT_3$ or $5HT_4$ receptors. However, ketanserin also has relatively high affinity for α-adrenergic and histamine H_1 receptors (Sanders-Bush and Mayer, 2001), actions which may also be relevant to ketanserin interactions with nicotine.

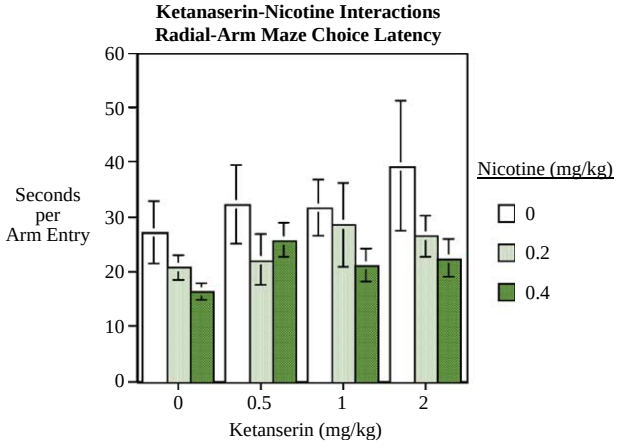

Fig. 2. Ketanserin interactions with nicotine and radial-arm maze response latency, seconds/arm entry (mean ± SEM).

Blockade of 5-HT_2 receptors is a common action of atypical antipsychotic drugs, such as clozapine and risperidone (Matsubara et al., 1993), though this mechanism may not be essential for antipsychotic efficacy (Wagner et al., 2005). These drugs can provide substantial relief from hallucinations of schizophrenia, their effect on cognitive function in schizophrenia is better than the older classic antipsychotics, like haloperidol (Weinberger and Gallhofer, 1997), but there is still need for better treatment for cognitive dysfunction of schizophrenia. There is considerable effort to develop co-treatments to improve cognitive impairment in patients with schizophrenia. Much of this effort is focused on nicotinic drugs since people with schizophrenia have abnormalities of nicotinic receptors (Court et al., 1999) and nicotinic agonists have been shown to improve cognitive function in patients with schizophrenia (Levin et al., 1996). The finding that the great majority of people with schizophrenia use tobacco (de Leon et al., 1995; Hughes et al., 1986) suggests that they may be self-medicating to alleviate their cognitive impairment. However, atypical antipsychotic medication may attenuate the effectiveness of nicotinic treatments for cognitive enhancement in schizophrenia inasmuch as the 5-HT_2 antagonist ketanserin was found to attenuate nicotine-induced memory and attentional improvement in the current and our previous study (Rezvani et al., 2005). There may be a need for special considerations in the development of nicotinic co-treatments for the cognitive impairment of schizophrenia to avoid or overcome the attenuation of the therapeutic effect by the antipsychotic drugs used.

Acknowledgements

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References

Addy N, Levin ED. Nicotine interactions with haloperidol, clozapine and risperidone and working memory function in rats. Neuropsychopharmacology 2002;27:534–41.

Adler LE, Hoffer LD, Wiser A, Freedman R. Normalization of auditory physiology by cigarette smoking in schizophrenic patients. Am J Psychiatry 1993;150:1856–61.

Aldridge JE, Levin ED, Seidler FJ, Slotkin TA. Developmental exposure to chlorpyrifos elicits behavioral alterations resembling animal models of depression and involving serotonergic mechanisms. Environ Health Perspect 2005;113:527–31.

Court J, Spurden D, Lloyd S, McKeith I, Ballard C, Cairns N, et al. Neuronal nicotinic receptors in dementia with Lewy bodies and schizophrenia: alpha-bungarotoxin and nicotine binding in the thalamus. J Neurochem 1999; 73:1590–7.

de Leon J, Dadvand M, Canuso C, White AO, Stanilla JK, Simpson GM. Schizophrenia and smoking: an epidemiological survey in a state hospital. Am J Psychiatry 1995;152:453–5.

Hughes JR, Hatsukami DK, Mitchell JE, Dahlgren LA. Prevalence of smoking among psychiatric outpatients. Am J Psychiatry 1986;143:993–7.

Kelly C, McCreadie R. Cigarette smoking and schizophrenia. Adv Psychiatr Treat 2000;6:327–31.

Levin ED, Wilson W, Rose JE, McEvoy J. Nicotine-haloperidol interactions and cognitive performance in schizophrenics. Neuropsychopharmacology 1996;15:429–36.

Levin ED, Simon BB. Nicotinic acetylcholine involvement in cognitive function in animals. Psychopharmacology 1998;138:217–30.

Levin ED, Rezvani AH. Development of nicotinic drug therapy for cognitive disorders. Eur J Pharmacology 2000;393:141–6.

Matsubara S, Matsubara R, Kusumi I, Koyama T, Yamashita I. Dopamine D1, D2 and serotonin2 receptor occupation by typical and atypical antipsychotic drugs in vivo. J Pharmacol Exp Ther 1993;265:498–508.

Newhouse PA, Potter A, Levin ED. Nicotinic system involvement in Alzheimer's and Parkinson's diseases: implications for therapeutics. Drugs Aging 1997;11:206–28.

Rezvani AH, Levin ED. Cognitive effects of nicotine. Biol Psychiatry 2001; 49:258–67.

Rezvani AH, Caldwell DP, Levin ED. Nicotine-serotonergic drug interactions and attentional performance in rats. Psychopharmacology 2005;179:521–8.

Ruotsalainen S, Sirvio J, Jakala P, Puumala T, MacDonald E, Riekkinen P Sr. Differential effects of three 5-HT receptor antagonists on the performance of rats in attentional and working memory tasks. Eur Neuropsychopharmacol 1997;7:99–108.

Sanders-Bush E, Mayer SE. 5-hydroxytryptamine (serotonin): receptor agonists and antagonists. In: Hardman JG, Limbird L, Goodman Gilman AG, editors. Goodman and Gilman's the pharmacological basis of therapeutics, 10th edition. New York: McGraw-Hill; 2001. p. 269–90.

Sumiyoshi T, Kido H, Sakamoto H, Urasaki K, Suzuki K, Yamaguchi N, et al. Time course of dopamine-D2 and serotonin-5-HT2 receptor occupancy rates by haloperidol and clozapine in vivo. Jpn J Psychiatry Neurol 1993; 47:131–7.

Wagner M, Quednow BB, Westheide J, Schlaepfer TE, Maier W, Kuhn KU. Cognitive improvement in schizophrenic patients does not require a serotonergic mechanism: randomized controlled trial of olanzapine vs amisulpride. Neuropsychopharmacology 2005;30:381–90.

Weinberger DR, Gallhofer B. Cognitive function in schizophrenia. Int Clin Psychopharmacol 1997;4(12 Suppl):S29–36.

White HK, Levin ED. Four-week nicotine skin patch treatment effects on cognitive performance in Alzheimer's disease. Psychopharmacology 1999; 143:158–65.

Wonnacott S, Irons J, Rapier C, Thorne B, Lunt GG. Presynaptic modulation of transmitter release by nicotinic receptors. In: Nordberg A, Fuxe K, Holmstedt B, Sundwall A, editors. Progress in brain research. Elsevier Science Publishers B.V. 1989. p. 157–63.