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Baclofen interactions with nicotine in rats: effects on memory

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Abstract

Nicotine has been shown in numerous previous studies to significantly improve memory on the radial-arm maze, yet the critical mechanisms underlying this effect are not fully characterized. Nicotine stimulates the release of a number of neurotransmitters important for memory function including (gamma-aminobutyric acid) GABA. The importance of nicotinic–GABA interactions regarding memory is currently unknown. The purpose of the current study was to determine the interactive effects of nicotine and the GABA agonist baclofen on working memory function as measured by choice accuracy in the radial-arm maze. Female Sprague–Dawley rats trained to asymptotic performance levels on a win-shift eight-arm radial maze task were used for assessment of nicotine–baclofen interactions. Low doses of baclofen improved memory performance while higher doses impaired it. Nicotine, as seen before, improved memory performance. Nicotine also significantly reversed the higher dose baclofen-induced deficit. These data show the importance of both nicotinic and GABA systems in working memory function and the interactions between these two transmitter receptor systems. This not only provides information concerning the neural bases of cognitive performance, it also lends insight into new combination treatments for memory impairment.

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

1. Introduction

Nicotine has been widely found to improve working memory function (for reviews, see (Levin and Rezvani, 2002; Levin and Simon, 1998)). However, nicotinic actions are complex and the neural bases for nicotine effects on memory have not been clearly delineated. Key to the complexity of nicotine actions on memory is its interaction with a variety of transmitter systems. Nicotine stimulates the release of numerous neurotransmitters including gamma-aminobutyric acid (GABA), glutamate, acetylcholine, dopamine, norepinepherine and serotonin (Wonnacott et al., 1989). These cascading effects of nicotine may play crucial roles in nicotine-induced memory improvement. As the primary inhibitory neurotransmitter in the brain, GABA is likely to be particularly important.

GABA systems have been shown to have important roles to play in memory function (Myhrer, 2003). Systemic administration of the GABA-B agonist baclofen induced deficits in water maze choice accuracy (Nakagawa et al., 1995). A variety of brain areas seem to be involved in this effect. Local infusions of baclofen to the basal forebrain, septum or mediodorsal thalamic nucleus has been shown to impair working memory in rats (DeSousa et al., 1994; Romanides et al., 1999; Stackman and Walsh, 1994).

GABA interactions with acetylcholine systems have been shown to be important for memory function. Both muscarinic and nicotinic cholinergic receptors appear to be involved. Baclofen-induced deficits in water maze choice accuracy were attenuated by the muscarinic cholinergic agonist oxotremorine (Nakagawa et al., 1995). The GABA–muscarinic interaction is complex. Baclofen effectively reversed the improvement in passive avoidance induced by the indirect cholinergic agonist physostigmine (Zarrindast et al., 1998). Nicotinic interactions with GABA

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regarding the behavioral expression of memory function still remain to be characterized. There is intriguing receptor binding and neurophysiological information showing functional nicotinic–GABA interactions in the hippocampus, an area critical for memory.

Chronic nicotine administration decreases hippocampal GABA-B expression (Li et al., 2002). Nicotinic receptors in the hippocampus facilitate output of GABA interneurons in the hippocampus which has complex control over the pyramidal cells in CA1, and are the main output pathway of the hippocampus. Nicotinic stimulation of hippocampal GABA neurons can cause net inhibition of pyramidal cells directly or through inhibition of inhibiting interneurons, causing disinhibition of these same neurons (Alkondon and Albuquerque, 2001). Nicotinic potentiation of hippocampal GABA neurons could also cause hyperpolarization of pyramidal cells, which would make them more responsive to subsequent stimulation, amplifying the later neural signals (Alkondon and Albuquerque, 2001).

The interaction of nicotine with GABA systems with regard to memory function has not yet been characterized. Based on this literature, we conducted the current studies to determine how nicotine effects on cognitive function are expressed under different levels of GABA receptor activation. We conducted the current project to determine the interactions of baclofen with nicotine with regard to working memory function because we hypothesized that the modulation of nicotinic mechanisms over GABA release (Zhu and Chiappinelli, 1999) would be important for its effects on memory. Baclofen effects on memory function were evaluated over a wide dose range with and without nicotine co-administration to determine possible differential interactions at low and high ends of the dose–response curve.

2. Methods

2.1. Subjects

Adult female Sprague–Dawley rats (N=12) were housed in groups of three in plastic cages with wood shavings. The rats lived in a vivarium (AAALAC-approved facility) immediately adjacent to the behavioral test facility, and were maintained on a reverse 12:12-h light–dark cycle with testing during the behaviorally active, dark phase. All of the rats had ad libitum access to water and were on a scheduled feeding regimen with one daily meal during the period of training and testing after drug administration. The rats were fed once daily after testing to keep their weight at approximately 85% of ad lib levels adjusted for growth. The rats grew from an average of 225 g at the onset of training to 265 g at the end of the drug phase. The experimental protocol was approved by the Duke University Institutional Review Committee for the use of animal subjects.

2.2. Radial-arm maze

The rats were trained for 18 sessions on the eight-arm radial maze. The eight-arm radial maze was made of wood and plastic and consisted of a central platform 50 cm in diameter, elevated 30 cm from the floor, with eight arms (10×60 cm) extending radially. Food cups for the reinforcers were located near the end of each arm. The maze was located in a room containing many extramaze visual cues. This training of 18 sessions brought the rats to asymptotic levels of memory performance. That is, they improved with training to a stable level of performance so that working memory function could be assessed. During the acquisition phase, the rats were tested on the radial-arm maze once per day (5 days/week). Each of the arms were baited with a reinforcer, and the rat was then placed in a plastic cylinder (30 cm in diameter and 20 cm high) on the central platform. To begin the session, the cylinder was lifted allowing the rat to move freely about the maze. Arm choices were recorded when the rat placed all of its paws into the arm. Since the arms were not rebaited during the session, only the first entry into an arm was rewarded. Subsequent entries into an arm previously entered were counted as errors. The session continued until either the rat entered all baited arms or 5 min elapsed.

2.3. Drug administration

After 18 training sessions, the rats entered the phase of drug testing. Acute nicotine–baclofen interactions were assessed over a wide dose range in two studies. In Experiment 1, memory effects of higher doses of baclofen (0.5 and 1 mg/kg) were characterized alone or in combination with nicotine (0.2 and 0.4 mg/kg). Then, in Experiment 2 in the same rats, memory effects of lower doses of baclofen (0.125 and 0.25 mg/kg) were characterized alone or in combination with nicotine (0.2 and 0.4 mg/kg). In both experiments, the saline vehicle was used as a control. Baclofen and nicotine doses were administered by acute subcutaneous injection 20 min before testing in a repeated measures Latin square counterbalanced design within each experiment whereby the animals received the dose combinations in different orders so that the order of injection was not confounded with dose. Nicotine ditartrate and baclofen HCl were obtained from Sigma (St. Louis, MO, USA). The drugs were mixed in saline. The volume of injection was 1 ml/kg. There were at least 2 days without testing between injections. The animals were found to perform at baseline levels during drug free testing between the two experimental drug challenge phases.

2.4. Statistical analysis

There was one measure of choice accuracy (entries to repeat) to index working memory performance. Response latency (seconds per entry) was also measured to determine

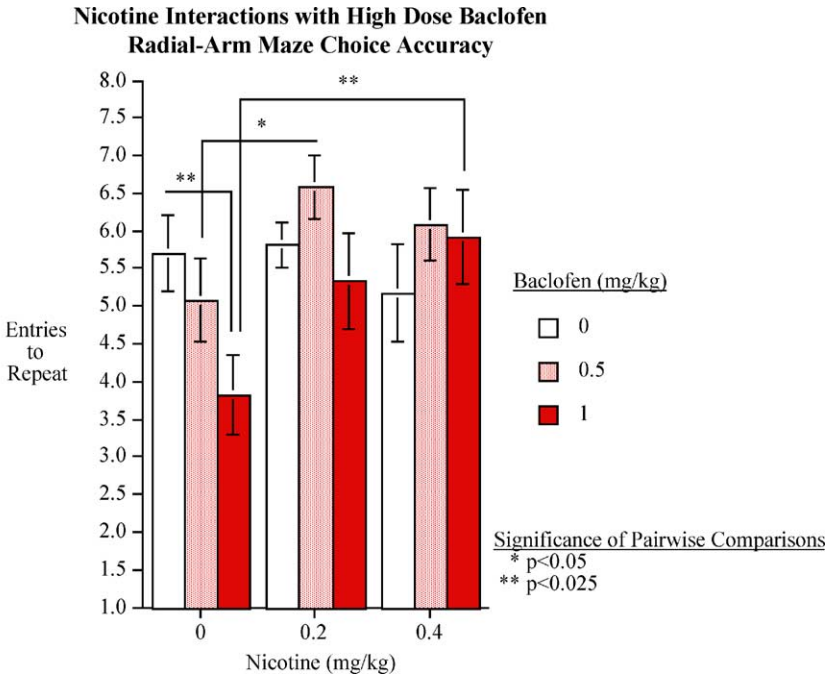

Fig. 1. High dose baclofen interactions with nicotine regarding choice accuracy (entries to repeat) in the radial-arm maze (mean±S.E.M.), N=12.

drug effects of sedation or hyperactivity. The drug effects were assessed by analysis of variance for repeated measures. The repeated measures were nicotine and baclofen drug doses. The study was conducted to test the hypotheses that nicotine and baclofen would each impact radial-arm maze performance and furthermore that nicotine and baclofen would modify each others' effects on radial-arm maze performance. Therefore, planned comparisons were made between vehicle control and the doses of either nicotine or baclofen alone to determine the impact of each drug on performance. To determine whether nicotine or baclofen would modify each others effect, planned comparisons were made between each drug alone with the combination of that drug and with the combination of two drugs. The threshold for significance was set at $p<0.05$ (two-tailed).

3. Results

3.1. Higher dose baclofen–nicotine interactions

Analysis of the main effect of nicotine showed significant choice accuracy improvement caused by either the 0.2 mg/kg ($F(1,22)=7.83$, $p<0.025$) and the 0.4 mg/kg ($F(1,22)=5.23$, $p<-0.05$) nicotine doses. However as shown in Fig. 1, this appeared to be due to nicotine reversal of baclofen-induced deficits. The higher dose range of baclofen caused a dose-related memory impairment with the 1 mg/kg baclofen dose causing a significant ($F(1,44)=6.61$, $p<0.025$) choice accuracy impairment relative to performance after saline injections. This baclofen-induced memory impairment was reversed by nicotine. When either the 0.2

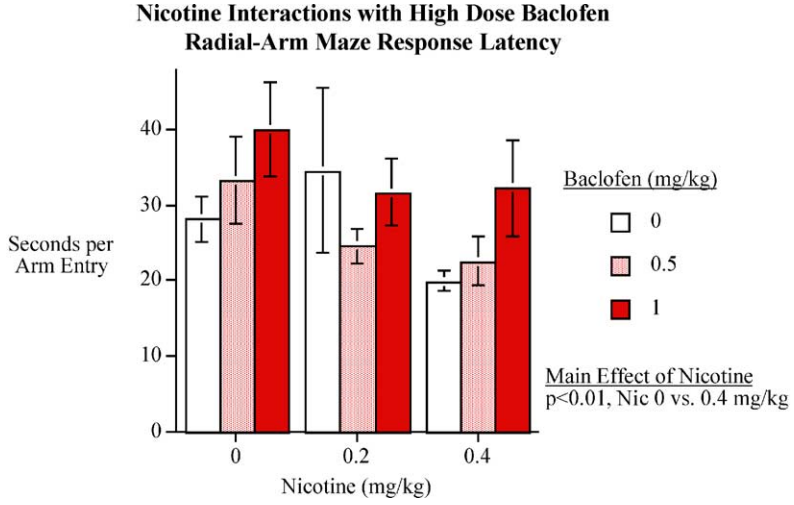

Fig. 2. High dose baclofen interactions with nicotine regarding response latency (seconds per arm entry) in the radial-arm maze (mean±S.E.M.), N=12.

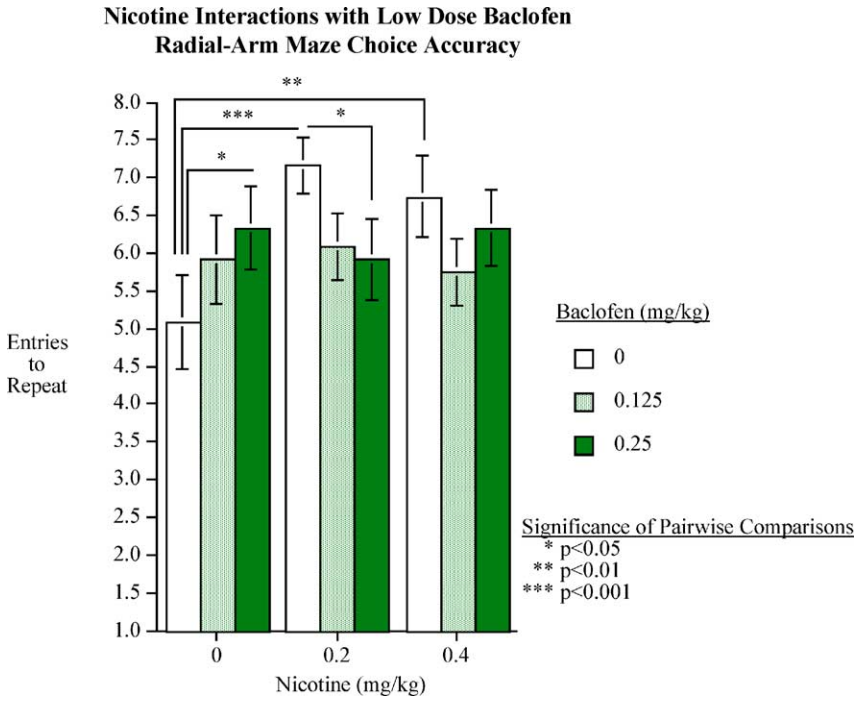

Fig. 3. Low dose baclofen interactions with nicotine regarding choice accuracy (entries to repeat) in the radial-arm maze (mean±S.E.M.), N=12.

mg/kg (F(1,44)=4.23, p<0.05) or the 0.4 mg/kg (F(1,44)=8.16, p<0.025) nicotine doses were given together with 1 mg/kg of baclofen, this significantly reversed this baclofen-induced memory impairment. Interestingly, when the 0.2 mg/kg nicotine dose was given together with 0.5 mg/kg of baclofen, it significantly (F(1,44)=4.23, p<0.05) improved choice accuracy relative to 0.5 mg/kg of baclofen alone even though this baclofen dose did not cause a significant impairment relative to saline.

This higher dose range of baclofen did not cause a significant increase in response latency (Fig. 2). Nicotine as a main effect (across baclofen doses) caused a significant reduction in response latency at the 0.4 mg/kg nicotine dose (F(1,44)=9.11, p<0.01) but not at the 0.2 mg/kg nicotine dose. Individual pair-wise comparisons of the individual dose combinations back to vehicle control did not detect any significant drug effects on response latency.

3.2. Lower dose baclofen–nicotine interactions

As shown in Fig. 3, the lower dose range of baclofen caused a dose-related memory improvement with the 0.25 mg/kg baclofen dose causing a significant (F(1,44)=4.82, p<0.025) choice accuracy improvement relative to performance after saline injections. In this experiment, nicotine by itself caused significant improvement in choice accuracy at both the 0.2 mg/kg (F(1,44)=13.40, p<0.001) and the 0.4 mg/kg (F(1,44)=8.58, p<0.01) doses. Baclofen

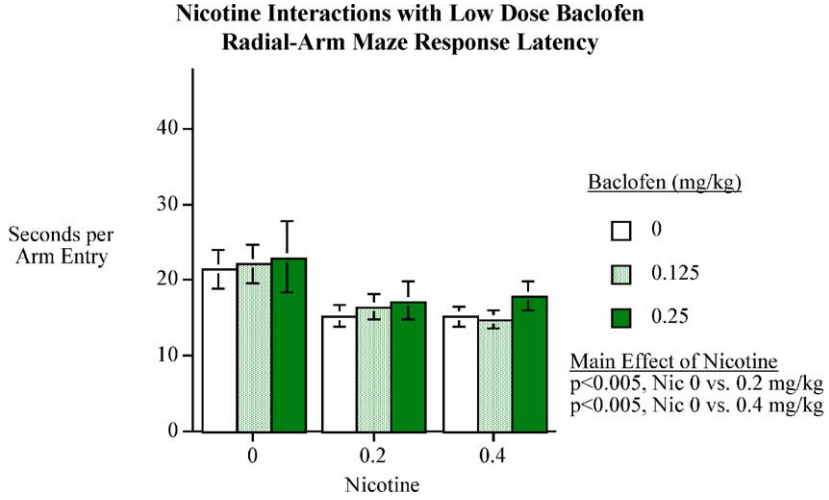

Fig. 4. Low dose baclofen interactions with nicotine regarding response latency (seconds per arm entry) in the radial-arm maze (mean±S.E.M.), N=12.

had a significant effect on the nicotine-induced memory improvement. The 0.25 mg/kg (($F1,44$)=p<0.05) baclofen dose significantly attenuated the memory improvement caused by 0.2 mg/kg of nicotine. The lower 0.125 mg/kg baclofen dose also had a trend in the same direction of attenuating the nicotine effect, but this was not quite significant (p<0.07). There were also trends of these baclofen doses attenuating the improvement caused by 0.4 mg/kg of nicotine, but these comparisons were not statistically significant.

Response latency (Fig. 4) was significantly reduced by both the 0.2 mg/kg (F(1,22)=11.39, p<0.005) and the 0.4 mg/kg (F(1,22)=13.17, p<0.005) nicotine doses as a main effect averaged over baclofen doses. There was no significant effect of low doses of baclofen on response latency in this experiment. The specific effects of each nicotine dose in the absence of baclofen co-administration also showed a significant quickening in response with both the 0.2 mg/kg (F(1,44)=8.00, p<0.01) and the 0.4 mg/kg (F(1,44)=8.33, p<0.01) nicotine doses causing significant reductions in latency relative to vehicle control. There was not any significant impact of baclofen on the nicotine-induced reduction in response latency.

4. Discussion

The GABA-B agonist baclofen had a biphasic effect on working memory function in the radial-arm maze. In the low dose range, baclofen caused a significant memory improvement, while in the high dose range baclofen impaired memory. Baclofen had important interactions with nicotine with regard to memory function.

As seen in previous studies (Levin and Simon, 1998), nicotine caused a significant memory improvement. This was reversed by low dose baclofen co-treatment. The high dose baclofen-induced memory impairment was reversed by nicotine co-treatment. Interestingly, memory improvement by nicotine alone was not seen in the high dose baclofen experiment. An analysis of the data was conducted to determine differences in control performance and response to nicotine given alone between the two experiments. There was not a significant difference in control choice accuracy performance between the two studies (p=0.31). Nicotine response was significantly higher in the low dose baclofen study than in the high dose baclofen study (p<0.05 for 0.2 mg/kg and p<0.025 for 0.4 mg/kg). It is possible that baclofen carryover effects in the higher baclofen dose study may have attenuated the expression of nicotine effects. Alternatively, the positive nicotine effects may have emerged more clearly with repeated administration so that it was more prominently expressed in the second study (low baclofen dose–response) than in the first study (high baclofen dose–response). Carryover effects of higher doses of baclofen also may have been responsible for the overall higher response latencies seen in the higher dose study vs. the lower dose study. There was no significant difference in the analysis of response latency after vehicle administration in the two studies (p=0.29). Interestingly, there was a significantly (p<0.05) longer latency with 0.2 mg/kg of nicotine given in the first (high baclofen dose–effect) than the second study (low baclofen dose–effect). As discussed above, this may be the result of differential carryover effects of the high and low baclofen doses in the two studies or the repeated nicotine exposure may have altered the expression of effect.

Previous studies have found that the central GABA agonist application has amnestic effects (Zarrindast et al., 2001). The finding that nicotine can reverse this effect may be related to nicotine-induced glutamate release, which could provide an excitatory counterweight to the inhibitory effects caused by excessive GABA actions. Nicotine has been shown to counteract the inhibitory effect of a GABA agonist applied to the hippocampus (Fujii et al., 2000). Nicotinic effects on hippocampal systems appear to involve both α7 and non-α7 nicotinic receptors (Alkondon et al., 1997; Fujii et al., 2000). In a practical sense, nicotine co-administration may provide symptomatic relief of the amnesia caused by GABA agonists given for epilepsy. Using nicotinic co-treatment of course rests on finding that nicotine at effective doses would not potentiate emergence of seizures. High doses of nicotine are proconvulsant when first given, but there is rapid tolerance for this effect and it is possible that chronic nicotine may provide further protection against seizures (Damaj et al., 1999).

The use of GABA-A agonist treatment has been found to be useful in attenuating the cognitive impairment of Alzheimer's disease (Maubach, 2003). Low doses of GABA-B agonists may be useful as well. The low dose baclofen-induced memory improvement in rats provides a useful laboratory model for this effect. While it is disappointing from a therapeutic point of view that nicotine and low-dose baclofen did not provide an additive effect of memory improvement, this important information will be valuable in further development of combination drug treatments for cognitive enhancement. The dose–effect function of a single drug improving memory function is nearly always an inverted-U in shape. Likewise, the combination of treatments with common mechanisms of action may also have a limit on the extent of improvement where either drug alone may cause improvement and thus further improvements may not be additive when the drugs are co-administered. In the case of baclofen and nicotine, the direct GABA stimulation by baclofen has a net common effect with nicotine-induced GABA release.

Why then does nicotine co-administration attenuate the higher dose baclofen-induced memory impairment? Nicotinic actions of stimulating GABA release would only serve to potentiate the GABA agonist-induced memory impairment. High doses of baclofen may provide excessive GABA stimulation such that further increases with nicotine-induced GABA release are without effect. Instead, other

nicotinic actions such as stimulated release of glutamate may become more prominent in the reversal of the baclofen-induced memory impairment.

Nicotine-induced reduction of the memory deficits caused by excessive GABA receptor stimulation from high dose baclofen is important because both nicotinic and GABA receptors modulate glutamate transmission in hippocampus, which in turn is a likely site of action of the newly approved Alzheimer's Disease drug memantine, a glutamate receptor antagonist. (Doraiswamy, 2002). Nicotinic interactions with GABA and glutamate may provide important new leads for treatments of Alzheimer's disease. Further research will help determine the promise of nicotinic receptor subtype selective drugs, which could adjust the GABA and glutamate interactions appropriately to reverse memory impairment. Further research will also help discern the role of nicotinic interactions with GABA and glutamate in the broader spectrum of cognitive functions including learning and attention.

Acknowledgement

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