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Idazoxan blocks the nicotine-induced reversal of the memory impairment caused by the NMDA glutamate receptor antagonist dizocilpine

Olga A. Timofeeva *, Edward D. Levin

Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, USA

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ABSTRACT

Rationale: Alpha2-adrenoreceptor (α_2-AR) antagonists have been shown to improve, while α_2-AR agonists impair cognitive function in subjects with functioning NMDA receptors (NMDAR). In subjects with inhibited NMDAR (a model of schizophrenia) α_2-AR agonists attenuate the cognitive impairments. The effect with α_2-AR antagonists remains unclear.
Objectives: We investigated the effects of the α_2-AR antagonist idazoxan on memory function in rats treated/not treated with NMDAR antagonist dizocilpine or a combination of dizocilpine and nicotine to clarify noradrenergic/cholinergic regulation of memory function.
Methods: Female Sprague–Dawley rats (n=12) were trained for food reward on the radial maze. Working and reference memory errors and response latency were assessed after injections of idazoxan (0.5, 1.0 mg/kg), dizocilpine (0.05 mg/kg), nicotine (0.2, 0.4 mg/kg) or vehicle, alone or in combination.
Results: Dizocilpine potently impaired memory. Nicotine (0.4 mg/kg) reversed this impairment. Idazoxan at the doses tested did not affect performance when given alone or with dizocilpine, but it did block the nicotine reversal of the dizocilpine-induced memory impairment. Three rats after 10–12 drug treatments developed limbic seizures. Our findings suggest that combination of drugs which block α_2-AR with nicotinic agonists in schizophrenia may prevent therapeutic effect of nicotinic agonists and increase risk for convulsive activity with repeated administration.

1. Introduction

Dizocilpine (MK-801), a glutamate NMDA receptor (NMDAR) channel blocker, originally described as an anticonvulsant and anesthetic drug was later found to cause cognitive deficits, psychotic symptoms, and a very specific pattern of widespread neurodegeneration. Such characteristics attract attention to this drug as a model compound in research of schizophrenia-like disorders. Clinical studies provide a convincing evidence that hypofunction of NMDAR is an important contributory process in the pathophysiology of schizophrenia (Newcomer and Krystal, 2001; Tsai and Coyle, 2002), and that disruption of memory function may be a manifestation of NMDAR hypofunction (Levin et al., 1998; Newcomer and Krystal, 2001). The mechanism of memory impairment in schizophrenics is poorly understood. However, it remains possible that interactions of neurotransmitter systems involved in cognition, particularly in the memory function, may bring insight about the neuronal circuitry underlying memory deficits, and may yield an understanding about pathophysiology of the behavioral impairments in schizophrenia and/or effective routes to its treatment.

Nicotine and other nicotinic receptor (nAChR) agonists have been consistently shown to improve different aspects of cognition in humans and in animals, including memory, attention, and learning (Brioni et al., 1997; Decker et al., 1995; Levin et al., 1998, 2002, 2006; Mansvelder et al., 2006; Nott and Levin, 2006; Warburton, 1992). A recent study (Weiss et al., 2007) demonstrated that both acute and chronic oral nicotinic treatments greatly improved deficits in cued and spatial learning of dopamine transporter knockout mice (representing pathology reminiscent of schizophrenia). Our previous study has shown that nicotine at the concentration of 0.4 mg/kg significantly attenuates dizocilpine-induced working memory deficits in rats (Levin et al., 1998). These findings are of particular importance because of increasing evidence of the involvement of the nicotinic receptor system in the pathophysiology of schizophrenia (Levin et al., 2006; Martin et al., 2004). For example, people with schizophrenia demonstrate high level of tobacco smoking and decreased number (Court et al., 1999; Freedman et al., 1995; Guan et al., 1999) and binding (Breese et al., 2000; Court et al., 1999; Durany et al., 2000) of nicotinic receptors in different brain areas, including the hippocampus.

The important role of the central noradrenergic system in regulation and modulation of cognitive functions is well known. It has been shown that elevating central noradrenergic activity, for instance by administration of alpha2-adrenoreceptor (α_2-AR) antagonists, such as idazoxan and atipamezole, improves performance in task assessing attention,

* Corresponding author. Box 3412, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC 27710, USA. Tel.: +1 919 681 4828; fax: +1 919 681 3416.
E-mail address: timofeev@duke.edu (O.A. Timofeeva).

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learning, and memory (Aston-Jones et al., 1991; Bunzey and Strupp, 1995; Devauges and Sara, 1991; Lapiz and Morilak, 2006; Sara and Devauges, 1989). In contrast, α_2-AR agonists administration that reduces noradrenergic transmission has deleterious effects on attention (Smith and Nutt, 1996) and target detection (Coull et al., 2004). Alpha2-AR antagonists enhance activity of noradrenergic neurons in the locus coeruleus and increase release of norepinephrine in the projection areas such as the neocortex and the hippocampus (Dennis et al., 1987; Fernandez-Pastor and Meana, 2002; Pudovkina and Westerink, 2005; Yavich et al., 2005), the regions that have been implicated in many cognitive functions. Moreover, α_2-AR antagonists increase, while α_2-AR agonists reduce the release of acetylcholine (ACh) in the medial prefrontal cortex (Tellez et al., 1997; Tellez et al., 1999) and glutamate/aspartate from cortical, hippocampal and thalamic synaptosomes (Kamisaki et al., 1992; Kamisaki et al., 1991). Recent investigations have also demonstrated that α_2-AR antagonists such as dexefaroxan can reverse cognitive deficits caused by UK 14304 (α_2-AR agonist), scopolamine (muscarinic receptor antagonist) and diazepam (Chopin et al., 2002). Atipamezole and RU-52583, another α_2-AR antagonists, ameliorate deficits associated with aging (Haapalinna et al., 2000) or the basal forebrain nuclei lesions (M'Harzi et al., 1997). However, cognitive deficits associated with NMDAR hypofunction, are attenuated with α_2-AR agonists, such as clonidine (Handa et al., 2000; Jentsch and Anzivino, 2004; Marrs et al., 2005). The effect of α_2-AR agonists on norepinephrine transmission and signaling in the brain is complex. These agents stimulate both pre- and postsynaptic α_2-AR. Activation of presynaptic receptors, located on adrenergic (autoreceptors) and non-adrenergic cells (heteroreceptors) would decrease the release of norepinephrine, ACh and glutamate in target areas (Dennis et al., 1987; Kamisaki et al., 1992, 1991; Pudovkina and Westerink, 2005; Tellez et al., 1997; Tellez et al., 1999), but direct stimulation of the postsynaptic α_2-AR would mimic the effect of norepinephrine. Several observations indicate that α_2-AR agonists can improve working memory, but under specific conditions, that challenge the prefrontal cortex function, such as during presentation of distracting stimuli (Arnsten and Contant, 1992). The mechanisms by which α_2-AR agonists improve cognition in subjects with inhibited NMDAR are unclear and need further investigation. The current study further examined α_2-AR involvement in regulation of cognitive functions, particularly, working memory, under condition of NMDAR inhibition. Working memory, unlike reference memory requires the integrity of the hippocampal formation (Barnes, 1988; Jarrard, 1978, 1986; Olton and Papas, 1979). The hippocampal formation has a peculiar very dense noradrenergic plexus in the dentate gyrus/hilar subregion pointing out a particular importance of the noradrenergic system in the hippocampal physiology, associated with memory processing. We used idazoxan, the drug widely utilized in pharmacological and behavioral studies, which was consistently shown to fully counteract the effects of α_2-AR agonists and indiscriminately and selectively block all four subtypes of α_2-AR (α_{2A}, α_{2B}, α_{2C}, and α_{2D}) (Clarke and Harris, 2002; Dabire, 1986; Michel et al., 1989). This approach would also allow, although indirectly, to explain cognitive improvement, caused by α_2-AR agonists in subjects with inhibited NMDAR.

The aims of the study were: 1) to clarify systemic effects of idazoxan alone or in combination with nicotine on working and reference memories in the 16-arm radial maze, 2) to evaluate effects of idazoxan alone or in combination with nicotine on memory deficits caused by NMDAR antagonist dizocilpine, and 3) to elucidate the involvement of noradrenergic transmission in regulation of the hipocampal-mediated working memory.

2. Methods

2.1. Subjects

Adult female Sprague–Dawley rats ($N=12$) were used in the present experiments, because it was shown that females are much more susceptible to NMDAR blockade than males (Honack and Loscher, 1993). The rats were kept on a reversed 12:12 h light-dark cycle. They were tested during morning hours, when they were active (dark phase of their daily cycle). Rats were housed in plastic cages in groups of three. They had *ad libitum* access to water, and were fed once daily, after testing, to keep them at 85% of *ad libitum* levels adjusted to growth. The experimental protocol was approved by the Duke University Institutional Review Committee for the use of animals.

2.2. Radial-arm maze (RAM)

The maze was located within a room with a variety of environmental cues: wall posters, an injection table, and the experimenter who always sat in the same place. The maze was made of painted black wood, and consisted of central platform 50 cm in diameter and 16 arms (10×60 cm). Each arm was partially flanked by a transparent Plexiglas to prevent the rats from jumping from arm to arm. Twelve out of sixteen arms were baited with reward (1/2 piece of Kellogg's Froot Loops) placed in food cups located in the end of each arm. Each rat had a unique pattern of baited and unbaited arms that remained constant throughout the training and testing periods. The rats underwent 20 training sessions (twice a week), which brought them to a stable level of performance so that working memory function could be assessed. Each session began with placing a rat in the central platform in a plastic cylinder. In 10 s the cylinder was removed allowing the rat to explore the maze. Each arm entered was recorded. Working memory errors were defined as a number of times the rat re-entered the initially baited arms. Reference memory errors were recorded each time the rat entered the unbaited arm. The session was completed either when the rat had entered all 12 baited arms, or when 10 min had expired. Latency, or the average amount of time per arm entry, was computed upon completion of the session.

2.3. Drug administration

The salt weights of the drugs were used in calculating doses. Nicotine tartrate, the nicotinic receptors agonist (0.0, 0.2 and 0.4 mg/kg), idazoxan hydrochloride, the α_2-adrenoreceptor blocker (0.0, 0.5 and 1.0 mg/kg) and dizocilpine ((+)-MK-801 hydrogen maleate), the non-competitive NMDA receptor antagonist (0.0 and 0.05 mg/kg) alone or in combinations (cocktail) were administered subcutaneously in a volume of 1 ml/kg, 20 min before the testing in a repeated measures Latin square counterbalanced design. Thus in each experiment the animals received dose combinations in different orders so that the order of injection was not confounded with the dose. All drugs were dissolved in bacteriostatic 0.9% sodium chloride, which also served as a control solution. Co-injection of each dose of nicotine, idazoxan and/or dizocilpine led to eighteen different possible combinations. Thus, each animal should receive 18 treatments. Animals were tested twice a week, on Tuesday and Thursday, thus there were 48 and 120 h between treatments, the intervals served to minimize possible carryover effects of the previous injection.

2.4. Statistics

All data are shown as mean±S.E.M. Repeated measures analysis of variance with planned comparison (SuperANOVA, SAS institute, Cary, NC) was used to assess the choice accuracy and response latency. The missing data (missing treatment tests when seizure occurred) were filled with values calculated as an average of values obtained in the rest of the animals receiving the same treatment. Differences were considered significant when $P<0.05$ (two-tailed). Particularly, we compared: 1) effects of nicotine and idazoxan, alone or in combinations, and effect of dizocilpine alone with that of saline, and 2)

Table 1
The treatment course and the seizure occurrence (marked as■) in the three animals

Rat#1	S+Il	S+Ih	S+Nl	S+Nl+Il	S+Nl+Ih	S+Nh	S+Nh+Il	S+Nh+Ih	D+Nh+Ih	D+S	D+Il+S■	■	D+Nl+S*	■	D+Nl+Ih■	D+Nh+Il*	D+Ih+S■	D+Nh+S		
Rat #7	S+Nh	D+Nh+Ih	D+Il	S+Nl	D+Nl+Ih	S+Nh+Il	S	S+Nl+Il	D+Nl+Il	D+Nh+S	S+Nh+Ih■	S+Il	D+Nl	S+Nl+Il	D+Nh+Il■	D+S	Ih+S	D+Nh+Il		
Rat #10	D+S	S+Ih	D+Nl+Il	S+Nh	D+Nh+Ih	D+Il+S	S+Nl	D+Nl+Ih	D+Nh+Il■	S	D+Ih+S	Nl+Il+S	D+Nh+S	Nh+Ih+S	Il+S	D+Nl	Nl+Ih+S■	D+Nh+Il		
Treatment #	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17	18	**19**		
Date	04/27	05/02	05/04	05/09	05/11	05/16	05/18	05/23	05/30	06/01	06/06	06/08	06/13	06/15	06/20	06/22	06/27	**07/13**		

S—Saline, N—Nicotine, I—Idazoxan, D—Dizocilpine, h—higher dose, l—lower dose.
*— Data not included in analysis.
■— No data obtained.

effect of dizocilpine alone with its combinations with nicotine and/or idazoxan.

3. Results

In the course of testing procedure, we observed unexpected behavioral seizures in 3 out of 12 animals. Animals #1, 7 and 10 developed seizures after 12, 12, and 10 treatments respectively. Seizures occurred suddenly either 20 min following drug injection at the very moment of plastic cylinder removal, when animal was allowed to explore the maze (7 cases), or before the treatment when animal was simply placed on the injection table (2 cases). Importantly, in all cases seizures started at the beginning of session, when animal surroundings were abruptly changed. Seizures began with freezing, staring, eye blinking, mouth foaming, proceeding then to head nodding, forelimb clonus, rearing, and finally to falling and clonic–tonic convulsions, lasting 2–5 min. The similarity of the behavioral manifestations in these rats to the unique behavioral pattern, produced by seizures in the hippocampal/amygdaloid complex in rodents (Racine, 1972; Timofeeva and Peterson, 1999) suggests that the behavioral phenomenon was a result of seizure activity originated in these structures. The treatment course and the days of seizure episodes in the three rats are shown in the Table 1. As it follows from the table, idazoxan was the only drug injected in all 7 cases, when seizures developed after drug administration. In animal #1 seizures occurred frequently and in the other two seizures developed rarely. We continued to test the rats according to the established previously design as shown in the Table 1. However, after the 18th treatment day the rats were given a 15-day drug-free rest period, during which the three rats were tested in the radial maze 3 times with saline to check for their condition and performance. No seizures were observed during this period. After this period the rats were tested again with one of their missing or unevaluated previously treatments. We included in analysis the following data obtained in the three animals: all data obtained before seizure episodes, data obtained after the 15-day drug-free period, and data obtained between seizures in animals #7 and 10, because their seizures were rare. To exclude the possibility that RAM data may have been affected by subclinical seizure activity, the results of each session were carefully examined on the presence of symptoms characteristic for learned behavioral automatism, which would result in increased errors of omission, dropped or uneaten bait reward, disorientation, impaired coordination, and changes in latency of performance. We found that none of the rats exhibited any of these symptoms. This makes it unlikely to expect the occurrence of subclinical seizures in the studied rats in the course of sessions. Because the observed seizures started before or at the very beginning of session, when animals' surroundings were abruptly changed, we suggest that the mechanism of their precipitation was situation-related. We also compared RAM performance in the animals treated with the same drugs/saline in the first versus the second half of the treatment course to find if prolong intermittent treatment procedure itself can affect memory score. We also found no difference in the

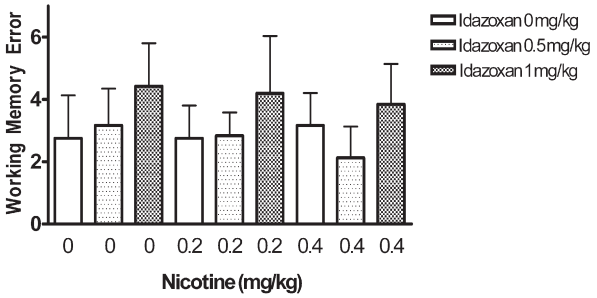

Fig. 1. Effects of systemic injections of idazoxan and nicotine alone or in combinations on working memory errors of rats in the 16 arm radial maze. The first bar represents performance errors after saline injections. *N*=12.

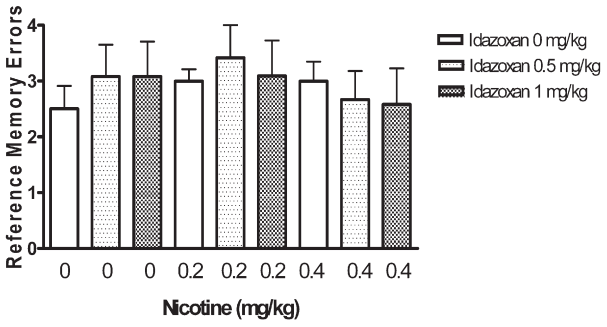

Fig. 2. Effects of systemic injections of idazoxan and nicotine alone or in combinations on reference memory errors of rats in the 16 arm radial maze. The first bar represents performance errors after saline injections. $N=12$.

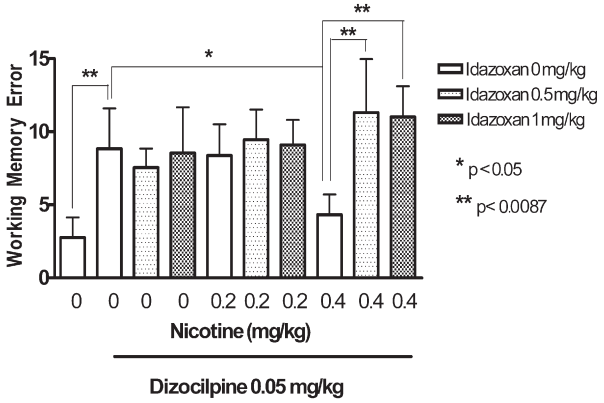

Fig. 4. Effects of systemic injections of different combinations of idazoxan, nicotine, and dizocilpine on working memory errors of rats in the 16 arm radial maze. The first bar represents performance errors after saline injections. $N=12$.

memory performance in the compared groups. However, there was an expected tendency of improvement in memory score in the second half of the treatment course. No changes in the latency of performance were observed. These observations evidence that RAM behavioral results were not affected by drug carry-over effects. It is important to note that during the entire observation period, which includes period of training and testing, the animals looked healthy, showed good behavioral activity, appetite, and steady body weight increase.

The average working and reference memory error score in rats treated with saline in the present study reached values of 2.7±1.37 and 2.5±0.4 respectively, which should be considered as a good memory performance. These values were significantly lower than values obtained during the first 4 days of training (9.2±0.4, $P<0.0003$ and 5.4±0.2, $P<0.0001$ for working and reference memory respectively, Student t-test, paired, two-tailed).

Effects of systemic administration of idazoxan or nicotine alone or in combinations on working and reference memory errors and on response latency are shown in Figs. 1–3, respectively. Our data demonstrate that compared with control vehicle, subcutaneous administration of idazoxan alone causes no significant changes in working memory errors at the studied doses ($F(1, 44)=0.035$, $p=0.85$, and $F(1, 44)=0.56$, $p=0.45$, Fig. 1), although there was a tendency of increasing errors at the higher doses (2.75±1.37, 3.16±1.17 and 4.41±1.39 errors at doses 0.0, 0.5, and 1.0 mg/kg, respectively). No statistically significant changes were found in reference memory errors (2.50±0.41, 3.08±0.57, 3.08±0.62 errors at 0.0, 0.5 and 1.0 mg/kg, respectively, $F(1, 44)=0.38$, $p=0.54$ and $F(1, 44)=0.38$, $p=0.54$, Fig. 2) as well as in response latency (14.40±0.82, 14.66±0.61 and 14.29±0.73 s/entry at 0.0, 0.5, and 1.0 mg/kg, respectively, $F(1, 44)=0.04$, $p=0.83$ and $F(1, 44)=0.009$, $p=0.92$, Fig. 3). No significant changes were observed also after systemic injection of nicotine at both doses of 0.2 and 0.4 mg/kg. However, our previous work (Levin et al., 1998) demonstrated improvement in working memory at the nicotine dose of 0.2 mg/kg. The absence of nicotine effect at the dose of 0.2 mg/kg in the present study most likely is due to the low level of performance errors in rats treated with control vehicle ("floor effect"). For example, in the mentioned above study nicotine at the dose of 0.2 mg/kg reduced average working and reference memory errors from 4.8 to 2.7 and from 4.2 to 3.8, respectively. In the present study in saline treated rats, these values were 2.75±1.37 and 2.5±0.4, respectively. Nicotine in the present study at both doses did not decrease response latency as was observed previously (Levin et al., 1998), this can also be due to a very fast performance ("floor effect") in the rats, treated with saline (14.4±0.81 s/entry). For example, in the previous study nicotine at the doses of 0.2 mg/kg and 0.4 mg/kg reduced latency from 20.6±1.9 to 15.2±1.1 and 14.9±0.9, respectively. Co-administration of nicotine and idazoxan also did not result in significant changes neither in working/reference memory errors nor in response latency.

Effects of dizocilpine alone and in combination with different doses of nicotine and/or idazoxan on working and reference memory errors and response latency are shown in Figs. 4–6. Our data demonstrate that dizocilpine at the dose of 0.05 mg/kg causes a significant increase in working memory errors compares to saline injection (2.75±1.37 vs. 8.83±2.75, $F(1, 44)=7.54$, $p=0.008$, Fig. 4). Increase in reference memory errors did not reach statistically significant level (2.50±0.41 vs. 4.33±0.67, $F(1, 44)=3.82$, $p=0.057$, Fig. 5). Dizocilpine also significantly reduced response latency (14.4±0.82 vs. 10.19±0.46, $F(1, 44)=11.46$, $p=0.001$, for saline and dizocilpine, respectively, Fig. 6).

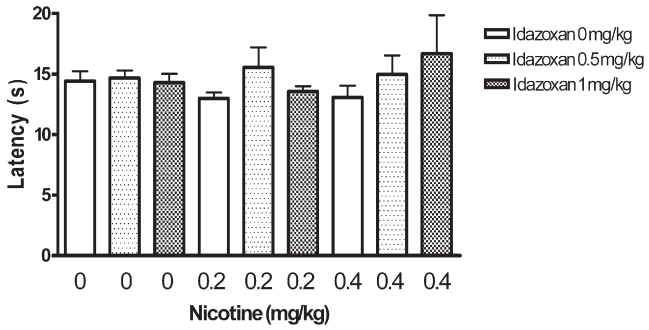

Fig. 3. Effects of systemic injections of idazoxan and nicotine alone or in combinations on response latency of rats in the 16 arm radial maze. The first bar represents performance errors after saline injections. $N=12$.

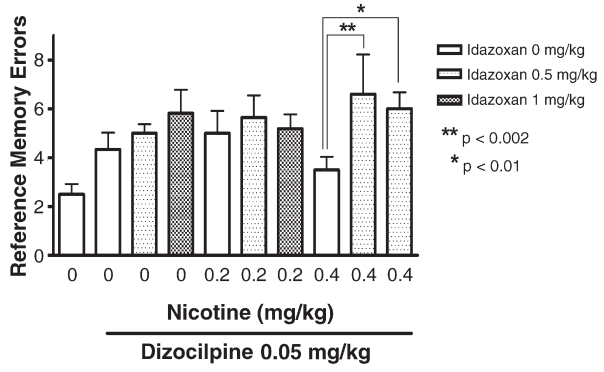

Fig. 5. Effects of systemic injections of different combinations of idazoxan, nicotine, and dizocilpine on reference memory errors of rats in the 16 arm radial maze. The first bar represents performance errors after saline injections. $N=12$.

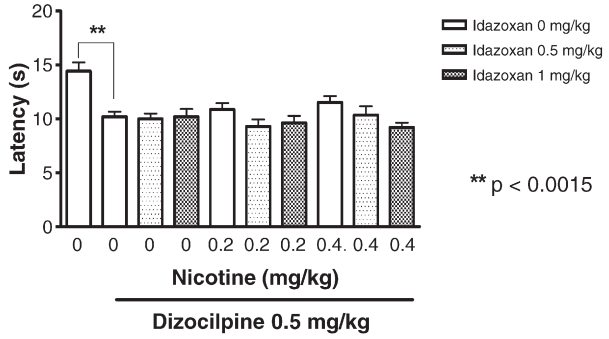

Fig. 6. Effects of systemic injections of different combinations of idazoxan, nicotine, and dizocilpine on response latency of rats in the 16 arm radial maze. The first bar represents response latency after saline injections. $N=12$.

Co-administration of dizocilpine with either doses of idazoxan or 0.2 mg/kg dose of nicotine diminishes neither working nor reference memory errors. No improvement in performance was observed when dizocilpine was co-administered with different combinations of nicotine and idazoxan. However, co-administration of dizocilpine with nicotine alone at the higher dose of 0.4 mg/kg resulted in substantial reduction of working memory errors (8.83±2.7 vs. 4.33±1.37, $F(1, 44)=4.13$, $p=0.004$, Fig. 4). Reduction in reference memory errors did not reach statistically significant level (4.33±0.67 vs. 3.5±0.52, $F(1, 44)=0.79$, $p=0.38$, Fig. 5). Importantly, addition of either dose of idazoxan reversed improving effect of 0.4 mg/kg of nicotine on dizocilpine-induced memory deficits. No statistically significant changes in response latency were observed with either treatment compares to dizocilpine alone (Fig. 6). This observation together with our previous report (Levin et al., 1998) evidence that nicotine-induced attenuation of memory impairment did not seem to be secondary to changes in motor activity.

4. Discussion

The present study demonstrates that dizocilpine at the dose of 0.05 mg/kg causes a significant memory impairment in the 16 arm radial maze, confirming our previous data (Levin et al., 1998) and the results of others (Hlinak and Krejci, 2006). We also confirmed our previous data (Levin et al., 1998) that nicotine co-treatment at the dose of 0.4 mg/kg, but not at the dose of 0.2 mg/kg significantly reduced this impairment. In the current study we also found that idazoxan at the studied doses did not affect memory performance when injected alone or with nicotine, it did not affect the dizocilpine-induced memory deficit but it did block the therapeutic effect of nicotine (0.4 mg/kg) reversing the dizocilpine-induced memory deficit. It has been proposed that in the radial maze tasks in which some arms are baited and some unbaited, the animal uses working memory to keep track of which arms it had entered as the trial proceeds and reference memory to avoid entries down arms that never contained food. Numerous studies report that the integrity of the hippocampal formation, its afferent and efferent connections is essential for working, but not reference memory operation. For example, it has been shown that lesions of the hippocampus and fimbria-fornix produce severe deficit in working memory, but do not have much effect on reference memory as was assessed with radial maze (Jarrard, 1978, 1986; Olton and Papas, 1979) and three-panel runway tasks (Kitajima et al., 1992). Similar results have been obtained in rats with hippocampal neuronal damage following transient forebrain ischemia (Davis et al., 1986). Intrahippocampal injections of benzodiazepine and muscimol (Ohno et al., 1992b), scopolamine or AMPA receptor antgonists (Ohno et al., 1992a), blockade of hippocampal nicotinic receptors (Ohno et al., 1993a), inhibition of hippocampal nitric oxide synthesis (Ohno et al., 1993b) produced selective impairment of working memory, without affecting reference memory. Although there is a consensus that hippocampus is a key structure for working memory operation, the real events are more complex, in which the hippocampus engages with other brain structures to mediate effective performance. In our study dizocilpine severely impaired working, but not reference memory, suggesting that blockade of NMDAR located in the hippocampus was most responsible for this effect. In addition, three out of the twelve animals in the course of treatments developed behavioral seizures typical in manifestation to those, originating in the hippocamal/amygdaloid complex (Racine, 1972; Timofeeva and Peterson, 1999). We consider the unexpected phenomenon as a very important event allowing further insight into the central mechanisms of the drug interaction, particularly at the level of hippocampal circuitry, known for its critical role in memory and high susceptibility to seizures.

High sensitivity of working memory to NMDAR inhibition, demonstrated in our studies, supports literature data on important role of NMDAR in memory (Shimizu et al., 2000), and learning (Huerta et al., 2000; Kullmann and Lamsa, 2007; Rampon and Tsien, 2000; Tsien et al., 1996). Long-term potentiation (LTP) and long-term depression (LTD), particularly in the hippocampal CA1 region and the dentate gyrus are regarded as cellular substrate of learning and memory. It is well known that axons of hippocampal principal neurons provide glutamatergic inputs to other principal neurons and local GABA-ergic interneurons as well as to GABA-ergic interneurons located in the lateral septum and the diagonal band of Broca. Hippocampal NMDAR are involved in the induction of LTP and LTD in principal neurons and interneurons (Grunze et al., 1996; Kullmann and Lamsa, 2007; Stelzer et al., 1994). Kullmann and Lamsa (2007) suggested that LTP in the hippocampal interneurons might provide a mechanism rapidly counteracting the increase in excitatory drive of pyramidal neurons that accompanies LTP at synapses on principal cells. Grunze et al. reported that LTP in the recurrent inhibitory circuit is more sensitive to NMDAR blockade than LTP in the excitatory pathway by an order of magnitude, suggesting that NMDAR antagonists may preferentially alter modulation of local circuit inhibition (Grunze et al., 1996). Reduction of the local inhibition leads to overstimulation of principal neurons via non-NMDAR (AMPA and Kainate) by excessive release of glutamate in the hippocampus and cortex (Moghaddam et al., 1997). In the same way, inhibition of NMDAR located on GABA-ergic interneurons in the septum and the diagonal band of Broca leads to disinhibition of cholinergic neurons projecting to the hippocampus (Giovannini et al., 1994) and cortex (Hasegawa et al., 1993; Kim et al., 1999) and subsequently to sustained increase in ACh release in those areas. The fact (Olney et al., 1991), that glutamate and muscarinic antagonists protect neurons against damage after systemic administration of NMDAR blockers, supports the hypothesis that hyperactivity of non-NMDA glutamate and muscarinic receptors is a critical component of the neurotoxic mechanism. Thus, available data point out that a failure of GABA-ergic inhibitory tone via blockade of MNDAR located on interneurons can be an important mechanism leading to a specific pattern of neuronal overstimulation and memory impairment.

The present study confirms our previous finding (Levin et al., 1998) that memory impairing effect of dizocilpine is significantly attenuated by concurrent injection of 0.4 mg/kg of nicotine, suggesting a functional interaction between nicotinic and glutamatergic systems. This fact also indicates that working memory can be operated with inhibited NMDAR and simultaneously activated nAChR. Series of studies (Bettany and Levin, 2001; Chambers et al., 1996; Levin, 2002) demonstrated a critical role of $\alpha7$ and $\alpha4\beta2$ nicotinic receptors in memory function, particularly those located in the hippocampus (Bettany and Levin, 2001; Felix and Levin, 1997; Levin et al., 2002) and basolateral amygdala (Addy et al., 2003). The presence of $\alpha7$ nAChRs on interneurons of the CA1 field of the rat hippocampus (Alkondon et

al., 1999; Frazier et al., 1998b; Ji and Dani, 2000; Jones and Yakel, 1997; McQuiston and Madison, 1998) as well as on the molecular layer and the hilar interneurons of the dentate gyrus (Frazier et al., 2003) has been well documented. These receptors mediate fast nicotinic cholinergic synaptic transmission and short bursts of action potentials on interneurons (Alkondon et al., 2000, 1999; Frazier et al., 1998a, 2003; Jones and Yakel, 1997) and short bursts of GABAergic IPSCs in various types of CA1 neurons and granule cells that receive innervation from the α7 nAChRs-expressing interneurons (Alkondon and Albuquerque, 2001; Frazier et al., 2003; Ji and Dani, 2000). The α4β2 nAChRs are also present in CA1 interneurons of different strata, including stratum radiatum interneurons (McQuiston and Madison, 1998; Sudweeks and Yakel, 2000). Activation of the α4β2 nAChRs triggers slowly decaying nicotinic currents as well as long bursts of action potentials in CA1 stratum radiatum interneurons, and evokes long bursts of GABAergic IPSP in CA1 neurons that receive innervation from the α4β2 nAChR-expressing interneurons (Alkondon and Albuquerque, 2001; Alkondon et al., 1999; McQuiston and Madison, 1998). In contrast, the principal neurons of the hippocampus and the dentate gyrus are generally unresponsive to applicatication of ACh in the presence of muscarinic receptor antagonist atropine (Frazier et al., 2003; Jones and Yakel, 1997) confirming prevalent location of the functional nAChR on interneurons of the hippocampal formation. Thus, available literature demonstrates that the excitability of the local interneurons is normally driven by both glutamatergic and cholinergic nicotinic inputs. In this regard it is important to mention our earlier finding of amnestic and pro-convulsive (at higher doses) effects of hippocampal infusions of nicotinic receptor antagonists (Felix and Levin, 1997) and a study of Dasheiff (1985), in which he demonstrated that ventricular, but not systemic injection of D-tubocurarine (non-specific nicotinic receptor antagonist) produces seizures. Together these findings suggest a critical role of nicotinic receptors in local inhibition, particularly in the hippocampal area, and imply that this neuronal pathway can regulate both seizure susceptibility and memory function. A recent study suggests that blockade of NMDAR results not only in the reduction of glutamatergic input to local interneurons but also in the reduction of the number of the α7 nAChRs (Kawai et al., 2002). Insufficient activation of local interneurons leads to hyperactivation of the principal hippocampal neurons. Importantly, exposure to nicotine activates available nicotinic receptors, and even increases their number (Kawai et al., 2002), thus restoring and maintaining local inhibition and signal processing in hippocampal neuronal networks, the area most critical in memory function. Nicotinic receptors efficiently bind nicotine even in the situation of elevated levels of endogenously released ACh because they have higher affinity to nicotine than to ACh. These events can explain the improving effect of nicotine on memory performance in rats co-administered with dizocilpine (Fig. 7).

We also found that systemic administration of idazoxan does not reduce memory impairment caused by dizocilpine. Moreover, co-administration of idazoxan with nicotine and dizocilpine reverses the improving effect of nicotine on memory performance. Numerous studies have demonstrated that in naïve subjects α_2-AR antagonists improve (Bunzey and Strupp, 1995; Devauges and Sara, 1991; Sirvio et al., 1993; Lapis and Morilak, 2006), while α_2-AR agonists reduce (Smith and Nutt 1996; Coull et al., 2004) cognitive functions. The effect of α_2-AR agonists seems to be opposite in the subjects treated with NMDAR blockers. For example, it was shown that α_2-AR agonists attenuate agitation and psychosis induced by anesthetic level of NMDAR antagonist ketamine in humans (Handa et al., 2000; Levanen et al., 1995; Newcomer et al., 1998), a low dose of α_2-AR agonist clonidine ameliorates visual attention and spatial working memory deficits in rats administered with phencyclidine (Jentsch and Anzivino, 2004; Marrs et al., 2005). Several studies demonstrated that α_2-AR antagonists dramatically increase (Kim et al., 1999; Tellez et al., 1997), while α_2-AR agonists significantly decrease ACh outflow in the rat prefrontal cortex (Tellez et al., 1997). It has also been shown that neurotoxic effects of NMDAR inhibition are blocked by systemic administration of several classes of receptor-specific

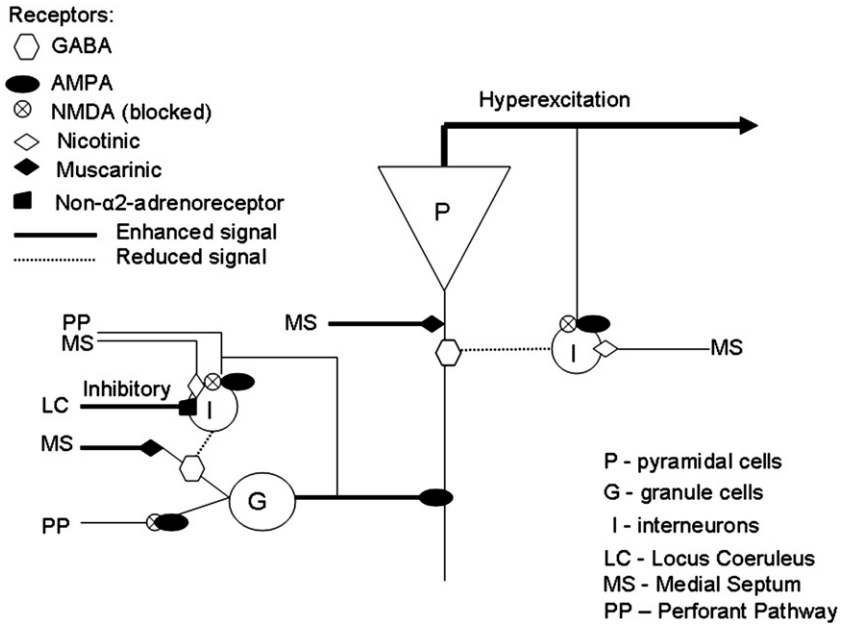

Fig. 7. Dentate gyrus-hippocampal local circuit neuronal interactions in the presence of dizocilpine and idazoxan. Dizocilpine blocks NMDAR (crossed circles). This compromises mainly local feedback inhibition of the hippocampal (P) and dentate gyrus (G) principal cells, and feedforward inhibition in the lateral septum (not shown). Increased release of ACh from the disinhibited medial septum-hippocampal pathway (MS) and glutamate from the principal neurons further contributes to the principal cells hyperactivation through muscarinic and AMPA receptors. Idazoxan and/or alarming surroundings activate the locus coeruleus (LC), inhibit feedforward interneurons (through released norepinephrine) in the dentate gyrus, thus disinhibiting further the granule cells (G), and increase release of ACh and glutamate in the cortico-hippocampal areas. The granule cells convey enhanced excitatory signal to the already hyperactivated pyramidal cells (P). Such situation can affect memory processing and lead to seizure activity, depending on the severity of disbalance of excitatory and inhibitory events.

transmitters, including agonists of $GABA_a$, α_2-AR, and antagonists of cholinergic muscarinic, and non-NMDA glutamate receptors (Farber et al., 1995; Jentsch and Anzivino, 2004; Kim et al., 1999; Olney et al., 1991). Thus, literature data point out that co-administration idazoxan with dizocilpine would further contribute to the hippocampal and cortical hyperexcitability by elevating ACh and glutamate release in these brain areas, while α_2-AR agonists would counteract dizocilpine-induced neuronal hyperactivation. There is an additional mechanism by which blockade of α_2-AR could contribute to hippocampal hyperexcitability: disinhibition of the dentate granule cells. It is well documented that the locus coeruleus innervation of the hippocampus has its densest terminal field in the subgranular/hilar region of the dentate gyrus (Blackstad et al., 1967; Loy et al., 1980; Oleskevich et al., 1989). A recent study (Brown et al., 2005) demonstrated that the locus coeruleus activation inhibit firing of all the dentate gyrus feedforward interneurons, while either enhancing or inhibiting subpopulation of feedback interneurons, and increasing as a net effect granule cell discharges. Another recent study (Knight and Harley, 2006) also found that idazoxan potentiated granule cell responses to the perforant path input in the dentate gyrus. It is conceivable, that systemic idazoxan by contributing to the dentate granule cell disinhibition, and cortico-limbic hyperexcitation reverses improving effect of nicotine associated with restoration of local inhibitory tone (Fig. 7). This scenario is supported by the observation of limbic seizures in 25% of our animals. Because seizures developed after 10–12 treatments, separated by prolonged (48–120 h) drug-free periods, the possibility of the development of chemical kindling and the possible role of each treatment drug in this process should be considered. As it follows from the literature analysis above, dizocilpine and idazoxan could have a proconvusive effect, because both drugs produce excessive release of glutamate and acetylcholine in the cortico-limbic areas. However, there is evidence that dizocilpine (MK-801) has strong antiseisure and antiepileptogenic properties (Kohl and Dannhardt, 2001; Loscher et al., 2003; Witkin et al., 1999). At the same time, a number of studies reported that α_2-AR antagonists contribute to kindling. For example, it was shown that amygdala kindling was accelerated by systemic administration of α_2-AR antagonists, idazoxan, yohimbine, and rauwolscine (Gellman et al., 1987; Shouse et al., 1996; Shouse et al., 2007). Conversely, α_2-AR agonists, such as clonidine and guanfacine, produce a dose-dependent delay of the amygdala electrical kindling in vivo (McIntyre and Giugno, 1988; Shouse et al., 2007) and in brain slices containing the dentate gyrus (Stringer and Lothman, 1991), and reported to be anticonvulsants in a number of other seizure models (Kunchandy and Kulkarni, 1987; Lazarova et al., 1983; Papanicolaou et al., 1982a,b). There is no evidence so far that nicotine can contribute to kindling. Observation from a variety of studies strongly indicates that muscarinic, but not nicotinic receptors contribute to the electrical or cholinergic kindling (Burchfiel et al., 1979; Cain, 1989; Meyerhoff and Bates, 1985). In addition, it was shown that nicotine, unlike carbachol, acetylcholine or serine, did not increase frequency of bicuculline-induced epileptiform discharges in the hippocampal CA3 area in vitro (Psarropoulou et al., 2003). Although nicotine facilitates release of acetylcholine and glutamate in different brain structures (Birthelmer et al., 2003; Fisher and Dani, 2000; Radcliffe and Dani, 1998; Radcliffe et al., 1999; Yamamoto et al., 2005), its low proconvulsive property can be result of simultaneous release of other neurotransmitters with anticonvulsive activity, such as GABA (Radcliffe et al., 1999) and norepinephrine (Azam and McIntosh, 2006; Fu et al., 1998; Rao et al., 2003; Sharp et al., 2004; Singer et al., 2004). Because nicotinic receptors are highly and predominantly expressed on hippocampal interneurons (Frazier et al., 1998a,b; Jones and Yakel, 1997; Sudweeks and Yakel, 2000) their inhibitory effect on pyramidal and granule cells can be prevailing (Radcliffe et al., 1999). Nevertheless, nicotine can induce seizures, but at much higher concentration, of 8–9 mg/kg. Typically systemic nicotine at such concentration causes immediate response, consisting of wild running and clonic–tonic convulsions (Damaj et al., 1999; Loscher et al., 2003), the manifestations very different from what we observed in our experiments. These data exclude role of nicotine in the seizure events observed in our experiments.

There are no literature reports that idazoxan can illicit seizures or kindling on its own. This, probably, is due to the ability of idazoxan to release norepinephrine, which unlike acetylcholine and glutamate has powerful antiseizure and antiepileptogenic properties (Giorgi et al., 2004). Although literature reports evidence that idazoxan can contribute to amygdala kindling, it is unlikely that the seizure phenomenon we observed in our experiments, was a result of kindling. The absence of progressive evolution of seizure severity, sporadic character of seizure provocation, development of spontaneous/reflex seizures and disappearance of seizures after the 15-day drug-free period bring evidence inconsistent with kindling. The fact that seizures were triggered in the rats by the removal of plastic cylinder from the maze arena or by placing them on the injection table, signifies the role of abruptly changing, alarming surroundings in seizure provocation, and points out that the observed seizures were reflex or situation-related. Scientific and clinical reports provide convincing evidence that the locus coeruleus is activated by novel or stressful or changing surroundings (Kitchigina et al., 1997; Sara et al., 1994; Vankov et al., 1995) and that the release of norepinephrine is especially prominent during situations of extreme vigilance, attention and stress. We can speculate that blockade of α_2-AR with idazoxan together with abrupt changes in surroundings can lead to hyperactivation of the locus coeruleus, disinhibition of the dentate granule cells, and increased release of ACh and glutamate in the hippocampus. This in turn can cause hyperactivation of the CA3 pyramidal neurons, which receive excitatory inputs from the granule cells and from the cholinergic medial septum, and are known to have a striking innate property for burst generation. Seizures began after 10–12 sessions: this is, probably, the time needed for reflex seizures (pairing between experimental surroundings and hyperactivation of the hippocampus) to develop. Such hypothesis would also explain negative effects of α_2-AR agonists on cognitive functions in the subjects with fully functioning NMDAR, and positive impact in the subjects with inhibited NMDAR. In the former case α_2-AR agonists would impair cognition by reducing release of ACh, glutamate and norepinephrine in the cortico-limbic areas. In the latter case, they will reduce hyperactivation in the same regions, which was produced by blockade of NMDAR. Another hypothesis on mechanisms of idazoxan reversing memory improving effect of nicotine can be associated with α_2-AR blockade. Nicotine increases norepinephrine release, which activates α_2-AR and thus improves memory performance. Idazoxan would prevent such effect by blocking α_2-AR. However, such events seems less likely to take place because nicotinic receptors in the hippocampus, the key structure for working memory, are predominantly located on interneurons. In addition there is a bunch of studies which demonstrated that α_2-AR are not significantly involved in the regulation of working memory (Grigoryan et al., 1994; Hiraga and Iwasaki, 1984; Kobayashi et al., 1995; Sirvio et al., 1992).

Thus, in our study using the 16-arm radial maze, we confirmed our previous findings that nicotine at the dose of 0.4 mg/kg reverses memory impairment produced by low dose (0.05 mg/kg) of dizocilpine. We also demonstrated that α_2-AR antagonist idazoxan does not significantly affect memory impairment caused by dizocilpine but fully antagonizes improving effect of nicotine. Current data and literature reports allow us to suggest the following events behind the observed phenomena (Fig. 7). Dizocilpine affects memory processing mainly by compromising local hippocampal inhibition via blockade of NMDAR located on interneurons. Nicotine counteracts this effect by restoring local inhibition through activation of nicotinic receptors on interneurons. Idazoxan reverses the effect of nicotine by disinhibiting dentate granule cells and increasing excitatory drive of principal neurons. This suggestion awaits experimental testing.

The NMDAR blockade with dizocilpine significantly impaired working memory. Inasmuch as this NMDAR blockade-induced memory impairment produced cognitive impairment similar to that of

schizophrenia, these results suggest that the heavy smoking by people with schizophrenia may serve to attenuate the impairment (albeit with great health risk from smoking). Nicotinic agonists under development for treatment of schizophrenia-associated cognitive impairment may have a similar beneficial effect. Importantly, since concurrent antagonism of α_2-AR blocks this effect, the administration of some antipsychotic drugs, which block a α_2-AR, may impair cognitive function in people with schizophrenia by blocking the therapeutic effect of nicotine. In addition, this drug combination could increase risk for convulsive activity with repeated administration.

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