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Chronic Memantine Treatment Prevents Short-Term Memory Impairment Caused by Conjoint Immunolesions of GABAergic and Cholinergic Medial Septal Neurons in Rats

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ABSTRACT. In the present study the effect of conjoint immunolesions of GABAergic and cholinergic medial septal (MS) neurons on spatial short-term memory is investigated and the effects of chronic memantine treatment in control and MS lesioned rats are evaluated. A total of 32 male outbred white rats were used in the present study. Rats were divided into Control and MS immunolesioned (conjoint lesion by GAT1-SAP and 192 IgG-saporin) groups and then into 2 subgroups injected i.p. with saline or memantine (5mg/kg daily for 13 days starting from the day of immunotoxins injection). Immunohistochemical studies showed that intraseptal injection of GAT1-SAP and 192 IgG-saporin significantly reduced GABAergic and cholinergic neurons in the MS as compared to contol rats. Behavioral study showed that memantine treated control rats, relative to saline treated rats, had a significantly lower level in the number of arms entered during the testing session. However, the groups did not differ in the level of alternation behavior. The results of behavioral study indicate that spatial short-term memory is affected by conjoint immunolesions of GABAergic and cholinergic MS neurons and the memantine treatment prevents short-term memory impairment caused by MS immunolesions. © *2016 Bull. Georg. Natl. Acad. Sci.*

Key words: spatial short-term memory, medial septum, immunolesion, memantine, rat

The medial septum (MS) is a subcomponent of the basal forebrain cholinergic system that mainly consists of an assemblage of cholinergic and GABAergic neurons. This projection is implicated in a variety of behavioral processes including memory and learning process [1]. Cognitive dysfunctions after MS lesions are mainly considered to be due to hippocampal deafferentiation and therefore may result in behavioral effects similar to those of hippocampal lesions. Lesions of this pathway as well as the hippocampal lesion produce deficits in a variety of cognitive tasks [2, 3]. Most basal forebrain functions

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were attributed to its cholinergic neurons and much attention was paid to the medial septal modulation of the hippocampus through cholinergic projections. Therefore, the MS lesioned animal is widely accepted as an animal model of Alzheimer's disease (AD) [4, 5]. However, an increasing body of evidence suggests that behavioral deficits after lesions of MS are not entirely due to destruction of cholinergic cells [6 - 9]. Recently, a more specific lesion technique was developed that allows selective lesioning of basal forebrain cholinergic or GABAergic neurons with immunotoxins - 192 IgG saporin [10] and GAT1-SAP [11]. Recently, Dashniani et al [12] showed that spatial short-term memory is affected only by electrolytic but not by 192 IgG saporin or GAT1-SAP lesions of the MS. Thus, as noted for other spatial memory tasks, deficits in performance caused by electrolytic MS lesions cannot be explained entirely by loss of cholinergic neurons. It may be suggested that the effects of electrolytic lesions can be caused by the lesion of the fibers of passage in the MS, but it is imposible to exclude, that memory impairing effect of electrolytic lesions of the MS is related to the combined lesions of the cholinergic and GABAergic septohippocampal projection neurons. Thus, it can be supposed that conjoint damage to MS GABAergic and cholinergic neurons will induce impairment of short-term spatial memory more severely than lesions of either population alone. Our study seeks to test this hypothesis in spatial alternation behavioral procedures using conjoint lesions of MS GABAergic and cholinergic neurons by immunotoxins.

Memory impairing effect of MS lesions may be caused by alteration in hippocampal glutamatergic transmission. It is well determined that both hypo- and hyperactivity of the glutamatergic system leads to hippocampal dysfunction: blockade of glutamatergic N-methyl-D-aspartate (NMDA) receptors leads to impairment of neuronal plasticity [13] while their overactivation leads to excitotoxic cell death due to calcium overload [14]. In several preclinical studies, uncompetitive NMDA receptor antagonist memantine enhanced performance in various animal models of AD, such as transgenic mice [15, 16], intracerebral ventricular infusion of â amyloid protein [17], and rats with entorinal cortex lesions [18]. Memantine prevents reference and working memory impairment caused by sleep deprivation [19]. In naïve animals, memantine failed to enhance [15, 20, 21] or disrupt [22] memory performance in some studies, but was reported to improve memory retention in healthy rats evaluated in a Morris water maze task [23]. It has been proposed that NMDA antagonists might prevent cognitive deterioration in MS lesioned rats. To address this question we evaluated the effects of memantine treatment on spatial alternation behavior in control and MS lesioned rats.

Material and Methods

Subjects. A total of 32 male outbred white rats weighing between 200 and 250 gm at the beginning of the experiment were used in the present study. The rats were housed in standard cages at a natural light/dark cycle and were tested during the light period. All animals were given access to food and water *ad libitum*. Rats were divided into Control and MS immunolesioned (conjoint lesion by GAT1-SAP and 192 IgG-saporin) groups.

Surgery. Rats were anaesthetized with i.p. injection of 4**%** chloral hydrate (9 ml/kg) and placed in a stereotaxic apparatus. All injections of 192 IgG-saporin (1 ìg/µl) and GAT1-SAP (325ng/µl) for immunolesion surgeries or mouse saporin (this product serves as a control for the immunotoxin) for control surgeries (Advanced Targetting System, San Diego, USA) were performed stereotaxically. Injection of GAT1-SAP were performed from the side by a 15 degree angle with the following coordinates AP- 0.4; M L -1.7; DV - 6.4 (0.5 µl; 0.05 µl/min). Injection of 192 IgG-saporin were performed bilateraly at two depths on each side: AP - 0.45; ML - 0.25; DV - 7.8 (0.3 µl; 0.05 µl/min) and DV - 6.2 (0.2 µl; 0.05 µl/min). After injection the needle was left in place for an additional 9 min and 6 min, respectively, to allow the toxin to diffuse from the

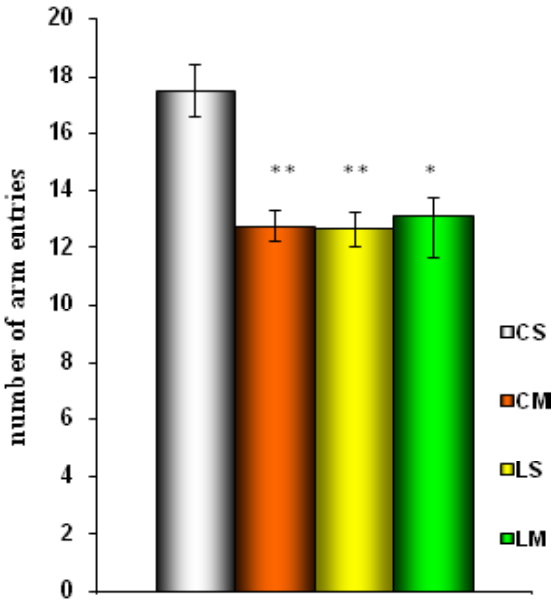

Fig. 1. Behavioral data (Mean ± SEM) from a single session of spontaneous alternation testing for control (CS - saline treated, CM - memantine treated) and MS lesioned (LS - saline treated, LM - memantine treated) rats. * P < 0.05; ** P < 0.01.

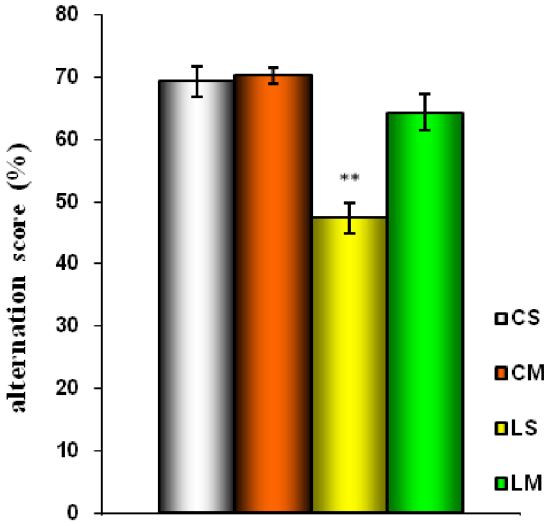

Fig. 2. Behavioral data (Mean ± SEM) from a single session of spontaneous alternation testing for control (CS - saline treated, CM - memantine treated) and MS lesioned (LS - saline treated, LM - memantine treated) rats. ** P < 0,001.

injection site. All injections were made with a 1-µl Hamilton syringe with a microinjection pump (CMA 402 Syringe Pump, Sweden). The rats were allowed to recover from the surgery for two weeks before starting the behavioral experiments. Control and immunolesioned groups of rats were divided into 2 subgroups: control rats injected i.p. with saline (n=8) or memantine (n=8) and immunolesioned rats injected i.p. with saline (n=8) or memantine (n=8). Memantine (5 mg/kg, i.p; Sigma Chemical Co., St. Louis, MO) or saline were given daily for 13 days starting from the day of immunotoxins injection. Intraperitoneal administration was chosen to avoid the variability in absorption associated with oral administration.

Spontaneous alternation behavior. Rats were trained in a four-arm plus-shaped maze with floor and walls made of black plexiglas. The arms of the maze (12.5 cm wide by 46 cm long by 7 cm high) extended radially from a central square platform (sides = 13 cm); the floor of the maze was positioned 0.7 m above the floor. Each rat was placed in the center of the maze allowed to transverse the maze freely for 15 min. The number and sequence of arms entered were recorded to determine alternation scores [24, 25]. An arm entry was defined as the entry with all four paws into one arm. The sequence of arm entries was recorded with a video camera. An alternation was defined as entry into four different arms on overlapping quintuple sets. Five consecutive arms choices within the total set of arm choices make up a quintuple set, e.g. a quintuple set consisting of arms choices A, B, A, C, B was not considered as alternation. Using this procedure percentage alternation was calculated as follows: (Actual alternation/possible alternation) × 100; possible alteration sequences are equal to number of arm entries minus four.

Histology. At the end of behavioral testing a random sample of rats from control (n=4) and immunolesioned (n=4) rats were killed and their brains collected in order to verify lesion effects. The immunotoxic (GAT1-SAP + 192 IgG-saporin) lesions of MS were verified by observing decreased Acetylcholintransferase (ChAT) and parvalbumine (PV) staining of the MS. The 20 ì thick coronal sections using freezing microtome were stained with ChAT and PV primary antibody and ABC Staining System. All necessary rea-

gents and buffers were received from Santa Cruz Biotechnology, Inc. (USA). Totally 6-10 sections of MS level within experimental and control animals were selected and was used to assess the effect of MS lesion on ChAT and PV-stained neurons. The sections were analyzed with a microscope Leica MM AF.

Statistical Analysis. Differences between groups were determined by one way ANOVA (SigmaStat statistical software). Two-sample *t*-test was used to compare immunohistological data between control and lesioned groups. All data are presented as mean ± standard error of the mean. Differences were considered significant when $p < 0.05$.

Results and Discussion

Immunohistochemical studies showed that intraseptal injection of GAT1-SAP and 192 IgG-saporin significantly reduced GABAergic and cholinergic neurons in the MS as compared to control rats ($p<0.01$).

The one way ANOVA for the number of arms entered during the testing session showed significant effect of group ($F_{3,31}$=5.962, P=0.003). Post hoc (Tukey Test) analysis showed a significant difference between the saline and memantine treated control rats (P =0.008). The significant difference revealed between the saline treated control and saline or memantine treated immunolesioned rats (P = 0.007; p =0.016, respectively). There was no significant difference between the saline and memantine treated immunolesioned rats (P=0.983).

Behavioral study showed that memantine treated control rats, relative to saline treated control rats, had a significantly lower level in the number of arms entered during the testing session. This data are in accordance with our previous study in which the chronic memantine treatment for 4 week reduces the number of arms entered by rats [26].

As shown in Fig. 2, immunolesion of MS significantly impaired SA performance. The one way ANOVA for spatial alternation score showed significant effect of group ($F_{3,31}$=20.449, P=0.001). Post hoc (Tukey Test) analysis showed a significant difference between the saline treated control and immunolesioned rats ($P < 0.001$), but there was no significant difference between the saline and memantine treated control (P =0.993) rats and between saline treated control and memantine treated immunolesioned (P = 0.454) rats. Memantine treatment causes improvement of spontaneous alternation performance; the difference between saline and memantine treated immunolesioned rats is significant ($P < 0.001$).

Interestingly, the findings showed that memantine treated control rats, relative to saline treated, had a significantly lower level in the number of arms entered during the testing session. However, these groups did not differ in the level of alternation behavior. It can be suggested that the reduced locomotor activity in MS immunole sioned rats are not responsible for reduced alternation scores. Furthermore, it can be assumed that the changes observed in short-term memory were due to the immunolesions of MS neurons.

Early studies using selective cholinergic lesions of basal forebrain neurons produced contradictory results. Studies examining spatial working memory using rewarded T-maze alternation could not find effects of cholinergic MS lesions [9, 27]. This study suggested that lesions limited to hippocampal cholinergic projections are insufficient for impairment. Study by Wrenn et al [10] reported deficits in working memory in the radial maze after intraventricular 192 IgG-saporin lesions. Intraventricular injections of toxin also damage cerebellar Purkinje cells and this experiment makes its result difficult to interpret. A few studies investigated the role of GABAergic septohippocampal projections in spatial memory. Recently, a more specific lesion technique was developed that allows for the selective lesioning of GABAergic neurons with GAT1-SAP. It is interesting to note that in our previous study selective lesion of GABA-ergic or cholinergic MS neurons did not affect spatial alternation [28]. According to the data obtained in the present study can be suggested

that conjoint damage to MS GABAergic and cholinergic neurons impairs short-term spatial memory more severely than lesions of either population alone.

In the present study we investigated the memory-enhancing potential of uncompetitive NMDA receptor antagonist memantine on spatial alternation impairments, induced by MS lesions. The effects of memantine were studied in relation to cognitive function in animal models of long-term memory, but rarely were tested in short-term memory paradigms. The effects of memantine on memory are found be dose- and time-dependent. To allow extrapolation of animal data to the clinical situation therapeutically relevant doses should be used in animal experiments. A therapeutically relevant dose in animals is the one that leads to plasma levels close to the therapeutic range in humans treated with therapeutic doses of memantine. Patients treated with 10-30 mg memantine per day, have plasma levels of 0.2-1.0 μM. Similarly, 1 μM plasma concentrations are achieved in rats after i.p. acute injection of 2.5-5.0 mg/kg [23]. Chronic treatment with memantine in male rats at dose which produces a plasma level within the therapeutic range was used to verify whether blocking NMDA receptors may impair or improve spatial short term memory. Our evaluation of memantine reveals that memantine treatment (5.0 mg/kg) causes improvement of short-term memory in adult rats assessed in spontaneous alternation task. These findings are in line with several investigations showing that memantine improved spatial working memory and modified the hippocampal synaptic plasticity markers in animal models of acute and repeated restraint stress [29]. Memantine prevents reference and working memory impairment caused by sleep deprivation [19].

Conclusion

Our results together with the short review of literature indicate that: (i) conjoint damage to MS GABAergic and cholinergic neurons impairs short-term spatial memory; (ii) the loss of septohippocampal cholinergic and GABAergic projections disrupt the function of the hippocampus to a sufficient extent to impair spatial short-term memory; (iii) Memantine treatment prevents short-term memory impairment caused by MS immunolesions.

ადამიანისა და ცხოველთა ფიზიოლოგია

მემანტინის ქრონიკული ზემოქმედება იწვევს მედიალური სეპტუმის GABA-ერგული და ქოლინერგული ნეირონების თანადროული იმუნოდაზიანებით გამოწვეული ხანმოკლე მეხსიერების დეფიციტის პრევენციას

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წარმოდგენილ ნაშრომში შეისწავლებოდა იმუნოტოქსინებით (GAT1-SAP და 192 IgG-საპორინ) მედიალური სეპტუმის GABA-ერგული და ქოლინერგული ნეირონების თანადროული დაზიანების ეფექტი სივრცით ხანმოკლე მეხსიერებაზე და მეხსიერების დეფიციტის პრევენციის შესაძლებლობა მემანტინით ქრონიკული ზემოქმედების პირობებში. ცდები ტარდებოდა მედიალური სეპტუმის იმუნოტოქსინებით დაზიანების მქონე და საკონტროლო ჯგუფის ცხოველებზე მემანტინის (იმუნოტოქსინების შეყვანიდან 13 დღის განმავლობაში დღეში 5მგ/კგ,) ან ფიზიოლოგიური ხსნარის ინტრაპერიტონიალურად შეყვანის პირობებში. იმუნოჰისტოქიმიური კვლევებით გამოვლინდა მედიალურ სეპტუმში იმუნოტოქსინების მიკროინექციის შედეგად GABA-ერგული და ქოლინერგული ნეირონების რაოდენობის სარწმუნო შემცირება საკონტროლო ჯგუფის ცხოველებთან შედარებით. ქცევით ექსპერიმენტებში გამოვლინდა, რომ საკონტროლო ჯგუფის ცხოველებში მემანტინით ქრონიკული ზემოქმედება მნიშვნელოვნად ამცირებს ლაბირინთის მკლავებში შესვლათა რაოდენობას, თუმცა სპონტანური მორიგეობის და შესაბამისად სივრცით ხანმოკლე მეხსიერების მაჩვენებლები ამ ჯგუფებში არ განსხვავდება. ქცევით ექსპერიმენტებში ასევე გამოვლინდა, რომ იმუნოტოქსინებით მედიალური სეპტუმის დაზიანება აუარესებს სივრცით ხანმოკლე მეხსიერებას, ხოლო მემანტინით ქრონიკული ზემოქმედება იწვევს სივრცითი ხანმოკლე მეხსიერების დეფიციტის პრევენციას.

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