Effect of an NMDA receptor agonist on T-maze and passive avoidance test in 12-week streptozotocin-induced diabetic rats

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Abstract:
This study examined behavioral effects mediated by NMDA (N-methyl-D-aspartic acid) receptors in 12-week streptozotocin (STZ)-induced diabetic rats. Effects of an NMDA receptor agonist on behavior in the open field test, passive avoidance test and T-maze were examined in control groups of rats and in rats with diabetes mellitus (DM). We have used 116 rats for experiments. Experimental type I diabetes was induced by a single intravenous injection of streptozotocin at a dose of 65 mg/kg, dissolved in citrate buffer. Stimulation with the NMDA receptor agonist at a dose of 15 mg/kg was performed 30 min before the experiments. In control rats, NMDA increased the number of crossing and rearings in the open field test, improved acquisition and consolidation processes and did not influence recall in the passive avoidance situation and was ineffective in the T-maze. Diabetes significantly inhibited locomotor and exploratory activity and profoundly impaired acquisition, consolidation and recalling in a passive avoidance, and significantly decreased working memory in T-maze. NMDA treatment of diabetic rats significantly improved memory in passive avoidance and T-maze. The NMDA receptor agonist increased locomotor activity in open field test. The obtained results suggested that stimulation of NMDA receptors had beneficial effects on learning and memory in type I diabetic rats.

Key words:
behavior, diabetes, NMDA, rat

Introduction

Diabetes mellitus (DM) is the most common serious metabolic disorder in humans. Diabetes is not one, but rather a group of related diseases characterized by hyperglycemia as a result of insulin shortage or insufficient insulin action, or both. The most common forms are type 1 diabetes, characterized by an immune-mediated destruction of pancreatic β-cells, leading to insulin deficiency, and type 2 diabetes, characterized by insulin resistance and relative insulin deficiency [15, 25, 37]. Both forms of diabetes are associated with long-term complications that affect the eyes, kidneys, heart, blood vessels and nerves [16, 23]. DM is associated with functional and structural alterations in the peripheral, as well as the central nervous system [11]. Moderate disturbances of learning and memory and complex information processes have been reported in both type 1 and 2 diabetic patients [3, 4, 9, 10]. Like in diabetic humans, a variety of neurophysiological functions are impaired in diabetic rats. At the molecular level, these impairments might entail distinct changes in glutamate receptor subtypes, in second-messenger system and in protein kinases. In diabetic rodents, more basic cognitive functions were af-
fected, especially learning and memory, and mental and motor function [4].

These cognitive deficits are associated with changes in hippocampal synaptic plasticity, including an impaired expression of long-term potentiation (LTP) [11, 21] and enhanced expression of long-term depression (LTD) [3, 22]. LTP and LTD are activity dependent modifications of synaptic strength, which have attracted considerable attention in the search for the cellular mechanisms of learning and memory [27, 32].

It is known that excitatory transmission in the brain is largely mediated by glutamate acting through different classes of receptors: ionotropic: AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), NMDA and metabotropic (mGluRs) [2]. In this study, the role of the glutamate NMDA receptors was of special interest. NMDA receptors are heteromeric glutamate-gated ion channels in the central nervous system (CNS) [2] and are formed by monomers of two families of homologous subunits NR1 and NR2A-D that are differentially expressed in the CNS [2, 11, 29].

Because NMDA receptors are believed to be involved in LTP, this receptor type seems to be very important for learning and memory [3, 11, 19, 34]. Learning deficits in streptozotocin (STZ)-diabetic rats were earlier shown to be paralleled by alterations in hippocampal synaptic plasticity [31]. Furthermore, a reduction of the activity of the NMDA receptor complex progresses after induction of diabetes. Gardoni et al. showed that one month STZ diabetes did not affect the autophosphorylation of NMDA receptor complex. In contrast, 4 months after induction of diabetes NR2A subunit immunoreactivity was impaired [14]. This fact seems to contribute to memory deficits in diabetic rats. In our previous experiments with NMDA receptor stimulation in 4-week DM, we observed that NMDA (15 mg/kg) given alone did not have any significant influence on motor activity in control rats except for the number of bar approaches, while in rats with 4-week DM, NMDA significantly increased motor activity in the open field test. In rats with experimental diabetes (4 week), NMDA increased acquisition, but it did not have any significant influence on consolidation and recall of passive avoidance responses. In control rats, NMDA (15 mg/kg) had no influence on a passive avoidance latency. In T-maze, NMDA increased working memory but only in diabetic rats [38]. Our previous experiments are difficult to explain because our data showed a positive effect of an NMDA receptor agonist on acquisition and working memory during 4-week DM. One month of STZ diabetes did not affect the NMDA receptor complex [14].

The aim of our study was to investigate the influence of NMDA, an agonist of the NMDA receptor on certain behaviors, such as learning and exploratory activity in rats 12 weeks after induction of type I diabetes in rats by STZ treatment.

**Materials and Methods**

**Animals**

The study was conducted on male Wistar rats weighing 250–300 g. They were housed in cages (55 × 40 × 20 cm), six animals per cage, in an air-conditioned (humidity 50–60%) and temperature-controlled (22°C) room under 12 h light/12 h dark cycle beginning at 7 a.m. The animals were fed a standard diet, food and water were freely available. The experiments were carried out between 8 a.m. and 12 a.m. Each animal was used only once and the same rat was not used in a different test.

The experimental procedures applied in this study were in compliance with the Board for Ethical Affairs and Supervision over Research on Animals and Individuals, Medical Academy of Białystok. Every effort was made to minimize the number of animals used and their suffering. All experiments were in accordance with the EU Directive 86/609/EEC and international guidelines on the ethical use of animals.

**Streptozotocin-induced type I diabetes mellitus**

Diabetes was induced by a single injection of STZ at 65 mg/kg (SIGMA, Germany) to rats via the tail vein. The STZ was dissolved in 0.1 M citrate buffer (pH adjusted to 4.5), and 5 days after the STZ injection, urine glucose level was measured by Tetra Phan Dia test (Pliva Lachema). Urine glucose level was determined in all STZ-injected animals. Twelve weeks after STZ administration, blood glucose concentration was measured using blood glucose test meter “super glucocard II” (Arkay, Japan).

Glucosuria and hyperglycemia (600 mg/dl) was observed in all animals. All experiments were carried out 12 weeks after STZ treatment.
Drugs

NMDA (Tocris, UK) at the dose of 15 mg/kg per rat was administered per os (po). Freshly prepared NMDA solution was given 30 min before the open field and T-maze tests or before the trial on the second day of the experiment in acquisition stage and on the 3rd day when recalling of the passive avoidance situation was tested. In consolidation of passive avoidance responses, NMDA was injected immediately after the trial on the 2nd day of passive avoidance experiment. The control rats received 0.9% NaCl (Polfa, Poznań). After the experiment, rats were anesthetized with chloral hydrate at the dose of 0.4 g/kg per rat and thereafter, they were killed by decapitation.

Behavioral testing

All experiments were carried out in a quiet, dimly lit room between 8 a.m. and 12 a.m. with each group equally represented at the times of testing. Each group comprised 10–12 rats. Rats were randomly allocated to treatment groups and were used only once. Passive avoidance responses were selected to estimate acquisition, consolidation and recall memory. Moreover, putative influence of the treatment on motor and exploratory activity was tested in open field, respectively.

Passive avoidance

The response was induced using the one-trial learning method of Ader et al. [1]. The apparatus consisted of a 6 × 25 cm platform illuminated with a 25 W electric bulb connected through a 6 × 6 cm opening with a dark compartment (40 × 40 × 40 cm). The floor of the cage was made of metal rods 3 mm in diameter, spaced by 1 cm. The investigation took advantage of the natural preference of rats to stay in dark compartments. The test lasted 3 days. On the first day, after 2 min of habituation in the dark compartment, rats were immediately removed.

Two similar trials, at an interval of 2 min, were carried out on the second day. After the first trial, rats were allowed to stay in the dark compartment for 10–15 s. In the second trial, when a rat entered the dark compartment, it received a foot shock (0.25 mA, 3 s) delivered through the metal rods. The presence of the passive avoidance was checked 24 h later. Rats were placed on the illuminated platform once more and latency to enter the dark compartment was measured, with the cut-off time at 300 s. To determine a possible effect of drug treatment on retrieval, according to the protocol proposed by Matthies [24], NMDA was administrated on the third day 30 min before the retention test. To determine a possible NMDA effect on consolidation, the drug at the dose of 15 mg/kg per rat was given immediately after induction of passive avoidance.

Locomotor and exploratory activity

The open field test was used to estimate locomotor activity in all groups of rats. The apparatus consisted of a square with 100 × 100 cm white floor, which was divided by 8 lines into 25 equal squares, and surrounded by white walls, 47 cm high. Four plastic bars, 20 cm high, were located at four different line crossings in the central area of the floor. A single rat was placed inside the apparatus for 1 min adaptation. Subsequently, crossings, rearings and bar approaches were counted for 5 min. NMDA (15 mg/kg) was given 30 min before the test.

T-maze

The procedure of T-maze test was described in detail elsewhere (modified by Braszko et al.) [5]. Briefly, a wooden runway, 68 × 8 × 7.5 cm was connected at the half of its length to the other runway, 48 × 8 × 7.5 cm constituting the base of the T-maze. The roof of the maze, made of wood, was appropriately hinged to longer runway and had a 12 × 7.5 cm window over the start area at the beginning of the base. The start area was closed by a hinged door. Aluminum plates (7 × 7 × 0.1 cm) were distributed on the floor of both runways at 12 cm intervals. The floor-facing surface of each plate was connected to a pressure-sensitive microswitch. The microswitch was connected to the microbulbs located on the upper surface of the roof. This system signaled the rat’s position inside the maze. Prior to the learning session the animals were deprived of food for 24 h. For learning trial, each rat was placed in the start area directly under the window. The bright light, emitted by a 60 W lamp located 20 cm above the window, prompted the animal to enter the dark parts of the maze (rats prefer the dark). The rats which did not start moving into the maze within 60 s, were excluded from the experiment. A piece of standard laboratory chow food was placed in a dish located 1 cm from the end of its side arm.
A correct choice was recorded when the rat approached the food, upon touching the food with its nose but without allowing the rat eating it, the animal was removed.

The procedure was repeated until 2 consecutive correct choices or 10 trials (whichever occurred first) were completed. After the learning session was carried out with one animal, the position of food was recorded, the maze was carefully cleaned, and the dish with food was placed in another arm. The rats, which did not reach the learning criterion (2 subsequent correct choices), were excluded from the experiment. Retention was tested after another 24 h of food deprivation following the feeding period. Each rat was given 5 test trials identical to the learning trials but carried out without food in the maze. During retention testing, a correct choice was the one in which the rat entered the maze side arm which previously, during the learning session, contained food. The percentage of the correct choices was calculated for each animal and taken as a measure of retention.

**Statistical analysis**

The statistical significance of the results was computed by two-way analysis of variance (ANOVA II) followed by Student’s *t*-test and by Newman-Keuls test, except for passive avoidance behavior assessed with Mann-Whitney ranking test. F-rations, degrees of freedom and *p* values are presented only for significant differences. For all comparisons, differences between particular groups with *p* equal to or lower than 0.05 were considered significant.

**Results**

The influence of NMDA on locomotor and exploratory activity of control and rats with type I DM in the open field test (Fig. 1)

We observed that NMDA at the examined doses significantly increased the number of crossed fields and

![Graph](image-url)
number of bar approaches but did not change the rearings in control rats.

STZ-induced diabetes significantly decreased locomotor and exploratory activity (number of crossed fields, rearings and bar approaches), while an NMDA agonist increased this activity in STZ-treated rats. The analysis of the number of crossed fields revealed significant treatments x diabetic interaction \([F(3, 33) = 5.91; p < 0.05]\). In the number of bar approaches, we observed significant interaction between treatment x diabetes \([F(3, 33) = 5.16; p < 0.05]\).

The effect of NMDA on acquisition, consolidation and recall of passive avoidance responses (Tab. 1)

![Fig. 2. The effect of NMDA in rats with DM on the working memory in the T-maze test. Rats received NMDA at the dose of 15 mg/kg ip. Control group received 0.9% NaCl. Columns represent the means ± SEM of the values obtained from 10 rats. *p < 0.05 vs. control DM (Newman-Keuls test)](image)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Acquisition (s)</th>
<th>Consolidation (s)</th>
<th>Recall (s)</th>
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<tbody>
<tr>
<td></td>
<td>M(Q1; Q2)</td>
<td>N</td>
<td>M(Q1; Q2)</td>
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<tr>
<td>NaCl</td>
<td>38.5 (29.5; 46.5)</td>
<td>12</td>
<td>27.5 (18.5; 44.5)</td>
</tr>
<tr>
<td>NMDA</td>
<td>51.5 (40.5; 73)*</td>
<td>12</td>
<td>52 (34; 77.5)*</td>
</tr>
<tr>
<td>DM 12/NaCl</td>
<td>27.5 (15.5; 30.5)*</td>
<td>12</td>
<td>16 (10; 29)*</td>
</tr>
<tr>
<td>DM 12/NMDA</td>
<td>100 (34; 225)* xxx **</td>
<td>13</td>
<td>84 (55.5; 115)** *</td>
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</table>

Rats received NMDA at the dose of 15 mg/kg ip. Control group received 0.9% NaCl. The retention latencies were expressed as the median (M) and interquartile range (Q1, Q3). The data were analyzed by two-tailed Mann-Whitney U-test. *p < 0.05, **p < 0.01, ***p < 0.001 vs. NaCl; \(xx\) p < 0.01, \(xxx\) p < 0.001 vs. DM 12; *p < 0.05, **p < 0.01 vs. NMDA.

The latency was shortened in diabetic rats vs. control group of rats. DM decreased acquisition of a passive avoidance responses (about 11 s). NMDA significantly prolonged time to entrance in the dark compartment in control and diabetic rats.

STZ-induced diabetes decreased consolidation of memory in a passive avoidance responses. Diabetes shortened the time spent on the illuminated platform by about 11 s. The NMDA significantly increased passive avoidance latency in rats with DM and in control.

STZ-DM significantly decreased recall in a passive avoidance test (about 19 s). The NMDA increased this process in rats with diabetes, but displayed no significant tendency in control.

The effect of NMDA receptor on working memory in diabetic and control rats in the T-maze test (Fig. 2)

In our examination, we observed that NMDA given at a dose of 15 mg/kg to STZ-induced diabetic rats significantly increased percent of the correct choices of the maze arm which previously contained food. At the tested dose, NMDA caused no change in memory in T-maze in diabetic rats. The analysis of the correct choices in T-maze revealed a significant main effect.

![Fig. 2. The effect of NMDA in rats with DM on the working memory in the T-maze test. Rats received NMDA at the dose of 15 mg/kg ip. Control group received 0.9% NaCl. Columns represent the means ± SEM of the values obtained from 10 rats. *p < 0.05 vs. control DM (Newman-Keuls test)](image)
of treatments x diabetes interaction \[F(3, 37) = 5.58; p < 0.05\].

**Discussion**

In our present experiments, we observed that NMDA, the agonist of glutamate receptor administered *ip* at the dose of 15 mg/kg significantly improved acquisition and consolidation and did not influence recall of passive avoidance situation. DM significantly impaired memory processes in both passive avoidance and T-maze tests. NMDA attenuated acquisition, consolidation and recall deficits induced by diabetes, and had positive effect on diabetes-evoked impairment of the number of correct entries in T-maze performance (at about 20%). These data complied with our previous results showing that NMDA given at the dose 15 mg/kg had significant influence on passive avoidance latency in 12-week STZ-diabetic rats but had no effect on 4-week STZ-diabetic rats [38]. This dose of NMDA was chosen on the basis of our unpublished data. In our experiments, the dose of NMDA of 15 mg/kg did not induce convulsions but it changed behavior in rats.

NMDA increased memory processes in this test. DM had no significant influence in 4-week STZ-diabetic rats on passive avoidance test, but impaired memory in 12-week diabetic rats in this test. Deficits of working memory in the T-maze test in 4-week diabetic rats and 12-week diabetic rats are similar, and in both NMDA increased working memory.

In animal models of diabetic pathology, such as the STZ-diabetic rats, spatial learning impairments have been reported [4]. STZ-diabetic rats also display deficits in cognitive tasks, such as performance in the Morris water maze [4, 8, 20]. In more complex learning tasks, such as an active and passive avoidance in T-maze, rats or mice with long-standing experimentally-induced diabetes consistently show performance deficits [3, 4, 12].

The variation in the result of these behavioral studies may be partially explained by differences in task complexity, animal models used and duration of diabetes [31]. Complex pattern of changes in synaptic plasticity has been observed in hippocampal slices from STZ-diabetic rats. Furthermore, STZ-induced diabetes in rats results in the altered function of NMDA and AMPA ionotropic and metabotropic glutamate receptors, which are implicated in learning and memory processes [6].

Evidence places NMDA receptors in the center of the learning process, and it has been noted that data are consistent across species including fish, birds, mammals and humans [34]. Despite some yet to be settled discrepancies, amnesia is observed when NMDA receptors are blocked during training in the water maze [34]. It is known that NMDA receptor antagonists can enhance or impair learning performance in animals [7, 24] and humans [33]. NMDA receptors are found at moderate concentrations in brain regions involved in learning and memory including the hippocampus and cortex [34, 36]. Induction of LTP requires NMDA receptors that are concentrated in the hippocampus, and administration of various NMDA receptor agonists improved cognitive ability while NMDA receptor antagonists impaired cognitive performance [34, 36].

It is known that expression of NMDA receptor subunits is decreased in the brain of STZ-diabetic rats [11, 34, 38]. Genetic studies showed that in rats with diabetes lasting 3 months, the immunoreactivity of NR2B subunits of NMDA receptor was reduced by about 40% [14]. In 2002, Gardoni et al. showed that one month of STZ-diabetes did not affect the NMDA receptor complex. On the contrary, 3–4 months after induction of diabetes in rats, NR2B subunit immunoreactivity, CaMKII and Tyr-dependent phosphorylation of the NR2A/B subunits of the NMDA receptor were reduced [14]. This fact seems to contribute to different memory deficits in 4-week and 11-week diabetic rats in the passive avoidance task. Flood et al. [12] observed that experimental diabetes (11 weeks) in mouse significantly decreased memory in shuttle box avoidance.

Morrison et al. [28] reported that shortly after treatment of rats with STZ (days), the diabetic state was accompanied by a substantial increase in sensitivity of the hippocampal slices to adenosine, owing to the loss of nucleoside uptake processes. The change in adenosine sensitivity persisting for a month might play a role in the memory deficits seen in diabetic rats [13, 28]. The loss of LTP maintenance in 6–8-week STZ-diabetic rats was a result of disruption of Ca\(^{2+}\)-dependent processes that modulate postsynaptic AMPA receptors during synaptic potentiation.

Furthermore, intensity and variety of electrophysiological abnormalities, structural changes and cognitive deficits may change in the course of diabetes.
Intensity of deficits of learning and memory may increase in the duration of diabetes and with intensification of pathological processes [38]. There is an interaction between ageing and diabetic dysfunction. It was discovered that memory deficits and changes in LTP increased along with age of rodents. It is known that LTP is impaired in 11–12-week STZ-induced diabetic rats, which is believed to be related to the cellular mechanisms of learning and memory [3, 20].

The changes in locomotor activity induced by NMDA may affect the data on passive avoidance and T-maze tests in our study. The number of entries, crossings and bar approaches was significantly decreased in STZ-diabetic rats, while NMDA increased locomotor activity in those rodents in the open field test. Some literature reports described that diabetes induced motor deficits in humans [17], and rats [25]. Decreased locomotor activity, also found in diabetic animals, was thought to be related to dysfunction of the striatal dopaminergic neurons [26]. Long-term STZ-diabetes in rats increased acute stress responses [35]. It is known that chronic stress-induced hedonia changes sensitivity of dopamine D2 receptors in the limbic forebrain [30]. Disturbances of dopaminergic and glutamatergic systems may lead to higher degree of cognitive impairments in STZ diabetic rats. It should also be noted that behavioral deficits in STZ-diabetic rats do not result from direct toxic effects of STZ on the brain [31].

In summary, diabetes profoundly impaired acquisition, consolidation and recall in passive avoidance paradigm and reduced the locomotor and exploratory activity. NMDA improved both memory deficits induced by diabetes in passive avoidance test and locomotor activity. In control rats, NMDA increased acquisition and consolidation in passive avoidance test, in open field test NMDA increased the number of crossing and bar approaches. In the T-maze test, NMDA increased working memory in diabetic rats.

On the other hand, many studies have indicated that the overactivation of ionotropic receptors such as NMDA receptor may mediate acute excitotoxic even [17, 18] and the chronic elevation of NMDA receptor activity has been demonstrated in a number of neurological disorders, including neurodegenerative states, such as Parkinson’s disease and Huntington’s chorea [19]. However, in our data we have not detected a negative impact of NMDA given at a dose 15 mg/kg in both groups of rats. Diabetes is characterized by hypofunction of NMDA receptor, so activation of this receptor would have a positive effect.

In conclusion, the administration of NMDA (15 mg/kg, po) can prevent diabetes-induced memory impairment in the passive avoidance and T-maze tests in 11–12-week diabetic rats.

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