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BEHAVIORAL EFFECTS OF D-AP7 IN RATS SUBJECTED TO EXPERIMENTAL HYPOXIA

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We investigated the effects of D-AP7 [D-(–)-2-amino-7-phosphonoheptanoic acid], a specific, potent antagonist of NMDA receptor on certain forms of behavior in control groups of rats and in rats submitted to hypoxia. D-AP7 given intracerebroventrically (*icv*) at a dose of 5 nmol was tested in the open field test, passive avoidance test and in elevated plus maze test.

D-AP7 did not significantly change the locomotor and exploratory activity, but it exhibited a tendency to enhance motility of rats in the open field test. It impaired the acquisition and did not influence the consolidation and retrieval in the passive avoidance situation. D-AP7 did not produce any significant effects in the elevated plus maze in rats which did not undergo hypoxia.

Short-term hypoxia (about 3 min) decreased the crossings, rearings and bar approaches in the open field test and impaired acquisition, consolidation and retrieval processes. It did not evoke any changes in elevated plus maze.

In hypoxia-treated groups of rats, D-AP7 enhanced locomotor and exploratory activity and it did not change the acquisition and retrieval processes. D-AP7 administrated before hypoxia impaired the consolidation in the passive avoidance test vs. D-AP7-treated group of rats. D-AP7 shortened the time spent in open arms and decreased the number of entries in open arms in hypoxia-treated groups of rats.

In conclusion, in hypoxia-treated groups of rats, D-AP7 enhanced motility, exhibited anxiogenic-like effect and impaired consolidation in passive avoidance.

Key words: *D-(–)-2-amino-7-phosphonoheptanoic acid (D-AP7), locomotion, passive avoidance, elevated plus maze, hypoxia, rats*

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INTRODUCTION

The majority of synapses in the mammalian central nervous system (CNS) use excitatory amino acids (EAA) as their neurotransmitters. L-glutamate is the dominant EAA in the brain [40]. Glutaminergic synaptic transmission is mediated by two major classes of receptors: ionotropic (iGluRs), ligand-gated ion channels for sodium and calcium and metabotropic (mGluRs), coupled with G-protein which modulate the production of second messengers [10, 23]. iGluRs are classified into subtypes according to their most selective agonists: AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate)/kainate and NMDA (N-methyl-D-aspartate) [36]. The NMDA receptors are widely distributed in the mammalian CNS with high densities in the superficial layers of the cerebral cortex, in the CA1 field and dentate gyrus of the hippocampus, in the granule cell layer of the cerebellum, in the striatum, septum, thalamus and spinal cord [11]. The widespread CNS distribution of NMDA receptors causes that these receptors are involved in physiological and pathological processes, such as learning and memory, neurotoxicity and hypoxic neuronal injury [8, 14, 38]. NMDA receptors play important role in cognitive performance [9]. The electrophysiological basis of memory processes is long-term potentiation (LTP). The NMDA subtype of glutamate receptors, in particular, is critically involved in LTP, a long lasting increase in synaptic efficacy which follows high-frequency stimulation [8]. It has been found that NMDA antagonists block induction of LTP in the CA1 region of hippocampus [25, 26].

D-(–)-2-amino-7-phosphonoheptanoic acid (D-AP7) is a specific, competitive antagonist of NMDA receptor. The antagonism is also selective, as kainate and glutamate responses are relatively unaffected [32]. In our previous study, D-AP7 did not affect locomotor and exploratory activity in the open field, did not influence consolidation or retrieval of passive avoidance and did not change object recognition regardless of rats [6].

Hypoxic conditions induce a release of several neurotransmitters, including EAA such as glutamate [28]. Increased level of extracellular glutamate and subsequent activation of NMDA receptors are primarily responsible for neuronal damage that occurs as a consequence of hypoxic episodes [33, 35, 39]. Episodes of hypoxia-ischemia cause changes in expression of NMDA receptors, viz. a decrease in a number of binding sites and an increase in affinity of the recognition site on NMDA receptor-ion channel complex [4, 21]. In our previous studies, we noted that hypoxia profoundly impaired some behavioral patterns in rats [5, 27].

In the present study, we tested the effect of blockade of NMDA receptor by D-AP7 on some behaviors: learning, exploratory activity and anxiety in rats subjected to experimental hypoxia.

MATERIAL and METHODS

Subjects

The study was conducted on white, male Wistar rats weighing 160–180 g. The animals were fed on "Murigran" standard diet and housed in plastic cages (50 × 40 × 20 cm), 10 animals per cage, in an air-conditioned (humidity 50–60%) and temperature-controlled (22° C) room under a 12 h light/ 12 h dark cycle beginning at 7.00 h. Food and water were freely available. All experiments were carried out between 8.00 and 12.00 h.

Surgery

The rats were anesthetized with chloral hydrate at a dose 0.4 g/kg *ip* and placed in a stereotaxic frame. The skull was cut in a middline and a hole was drilled 2.5 mm laterally and 1 mm caudally from the point of intersection of bregma and the superior sagittal suture on the right side of the head. A plastic cannula with a 0.7 mm external diameter was surgically implanted 5 mm below the skull surface. The injections were made using a Hamilton microliter syringe with a 0.3 mm external diameter. After implantation, rats were housed individually and allowed to rest for 7 days.

Drugs

D-(–)-2-amino-7-phosphonoheptanoic acid (D-AP7, Tocris Cookson, UK) was administered through a cannula into the lateral ventricle of the brain (*icv*) at a dose of 5 nmol per rat in the volume of 5 µl [6, 41]. Control rats received saline (0.9% NaCl, Polfa, Poznań, Poland) *icv* in the volume of 5 µl.

Amnesia induced by hypoxia

Hypoxia was produced by placing rats in a glass chamber flushed with a mixture of 2% O_2 in N_2 [3] till respiratory arrest, after which they were immediately transferred to air. The hypoxia was induced

30 min before examining animals' performance in the open field test and elevated plus maze test. In the passive avoidance situation, hypoxia was induced on the second day 30 min before training, or immediately after completion of training, or 30 min before it on the third day, when we determine effect of hypoxia on acquisition, consolidation or retrieval, respectively.

Behavioral tests

Passive avoidance response training

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 at 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, the 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 the latency to enter the dark compartment was measured, with the cut-off time of 300 s. To determine the effect of drug treatment on retrieval, according to the protocol proposed by Matthies [20], D-AP7 was administered on the third day 30 min before retention test. To determine D-AP7 effect on consolidation, the drug was given on the second day immediately after completion of induction of passive avoidance or 30 min before it to determine D-AP7 effect on acquisition. The rats were subjected to hypoxia immediately after the injection of D-AP7.

Locomotor and exploratory activity

The open field test was used for estimation of locomotor activity 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 wall, 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 of adaptation. Subsequently, crossings, rearings, and bar approaches were counted manually for 5 min. D-AP7 was given 30 min before the test and then immediately the rats were underwent hypoxia.

Elevated plus maze

The maze (constructed of grey colored wooden planks) consisted of two open arms, 50 cm (length) × 10 cm (width) and two closed arms, 50 cm (length) × 10 cm (width) × 40 cm (height), covered with a removable lid, such that the open or closed arms were opposite to each other. The maze was elevated to a height of 50 cm from the floor. Fifteen minutes after the injection, a naive rat was placed for 5 min in a pretest arena (60 × 60 × 35 cm, constructed from the same material) prior to exposure to the maze. This step allows for the facilitation of exploratory behavior. The experimental procedure was similar to that described by Pellow et al. [31]. Immediately after the pretest exposure, rats were placed in the center of the elevated plus maze facing one of the open arms. During the 5 min test period, the following measurements were taken: the number of entries into the open and closed arms and the time spent in the open and closed arms. An entry was defined as the entry with all four feet into one arm. An increase in open arm entries and increase in time spent in open arms is indicative of potential anxiolytic activity, as rats naturally prefer the closed arms. D-AP7 was given 20 min before pretest and then immediately the rats underwent hypoxia.

Statistical analysis

The statistical significance of the results was computed by one-way analysis of variance (ANOVA) followed by Student's *t*-test and by Newman Keuls tests, except for passive avoidance behavior which was assessed with Mann-Whitney ranking test. F rations, degrees of freedom and p values are reported only for significant differences. In all comparisons between particular groups a probability of 0.05 or less was considered significant.

This work was approved by the Ethical Committee of Medical Academy in Białystok.

RESULTS

The effect of D-AP7 on locomotor and exploratory activity of control and hypoxia-treated rats in the open field test (Fig. 1)

D-AP7 did not significantly change the crossings, rearings and bar approaches, but it exhibited a tendency to enhance motility of rats in the open field test. Rats subjected to hypoxia showed profound impairment of locomotor and exploratory activity. D-AP7 in hypoxia-treated group increased the number of crossed fields, rearings and bar approaches vs. hypoxic control group of rats.

The effect of D-AP7 on acquisition of passive avoidance in control and hypoxia-treated rats (Fig. 2a)

D-AP7 significantly shortened the latency in rats. Hypoxia also shortened the time spent on the illuminated platform. D-AP7 in hypoxia-treated rats did not significantly change the latency to enter the dark compartment.

The effect of D-AP7 on consolidation of passive avoidance in control and hypoxia-treated rats (Fig. 2b)

D-AP7 did not change the time spent on the illuminated platform. The latency was shortened in hypoxia-treated group of rats. D-AP7 administrated before hypoxia significantly shortened the latency of entrance to dark compartment vs. D-AP7-treated control group of rats.

The effect of D-AP7 on retrieval of passive avoidance in control and hypoxia-treated rats (Fig. 2c)

Hypoxia shortened the latency in rats. D-AP7 did not significantly change the time spent on the platform in groups of rats submitted and not submitted to hypoxia.

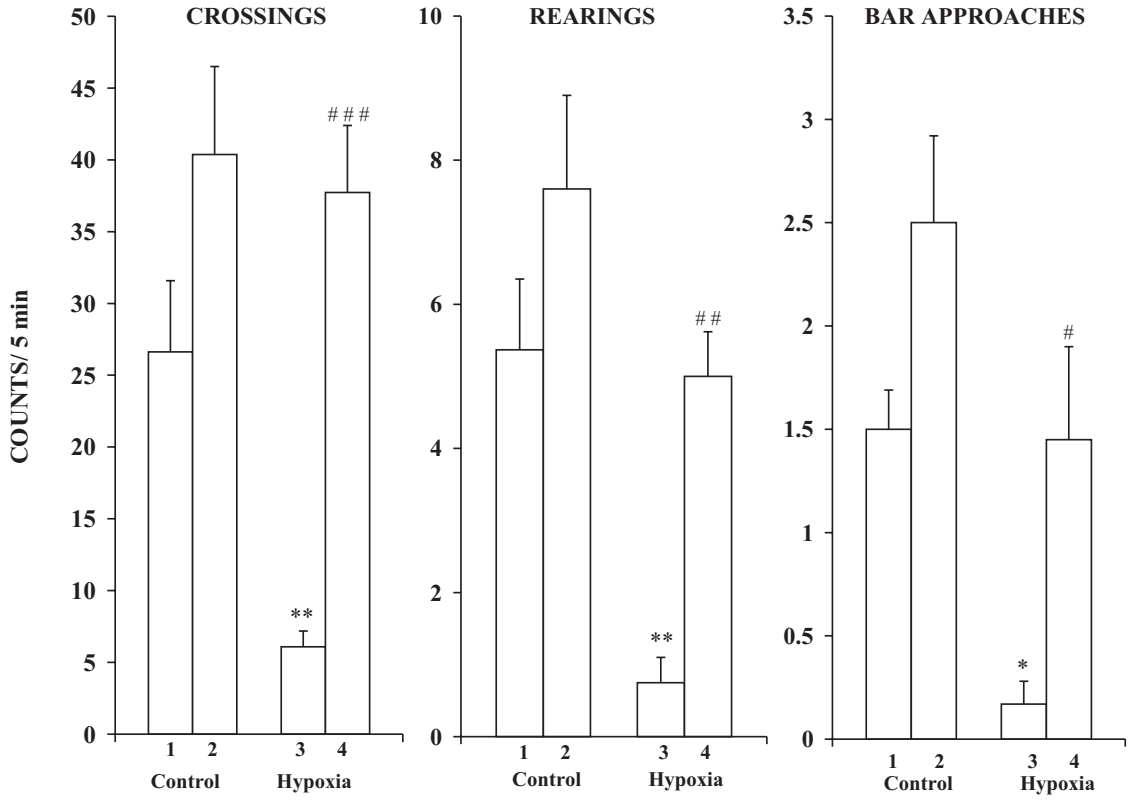

Fig. 1. The effect of D-AP7 on number of crossings, rearings bar approaches in the open field in control (groups 1–2), and hypoxia-treated rats (groups 3–4). Columns represent means ± SEM of the values obtained from 8–12 animals. 1) saline (0.9% NaCl 5 µl *icv*); 2) D-AP7 (5 nmol *icv*); 3) saline (0.9% NaCl 5 µl *icv*) + hypoxia; 4) D-AP7 (5 nmol *icv*) + hypoxia. Crossings $F(3.35) = 15.309$; ** $p(1–3) < 0.01$; ### $p(3-4) < 0.001$. Rearings $F(3.35) = 14.128$; ** $p(1–3) < 0.01$; ## $p(3–4) < 0.01$. Bar approaches $F(3.35) = 8.817$; * $p(1–3) < 0.05$; # $p(3–4) < 0.05$ (ANOVA, Newman-Keuls tests)

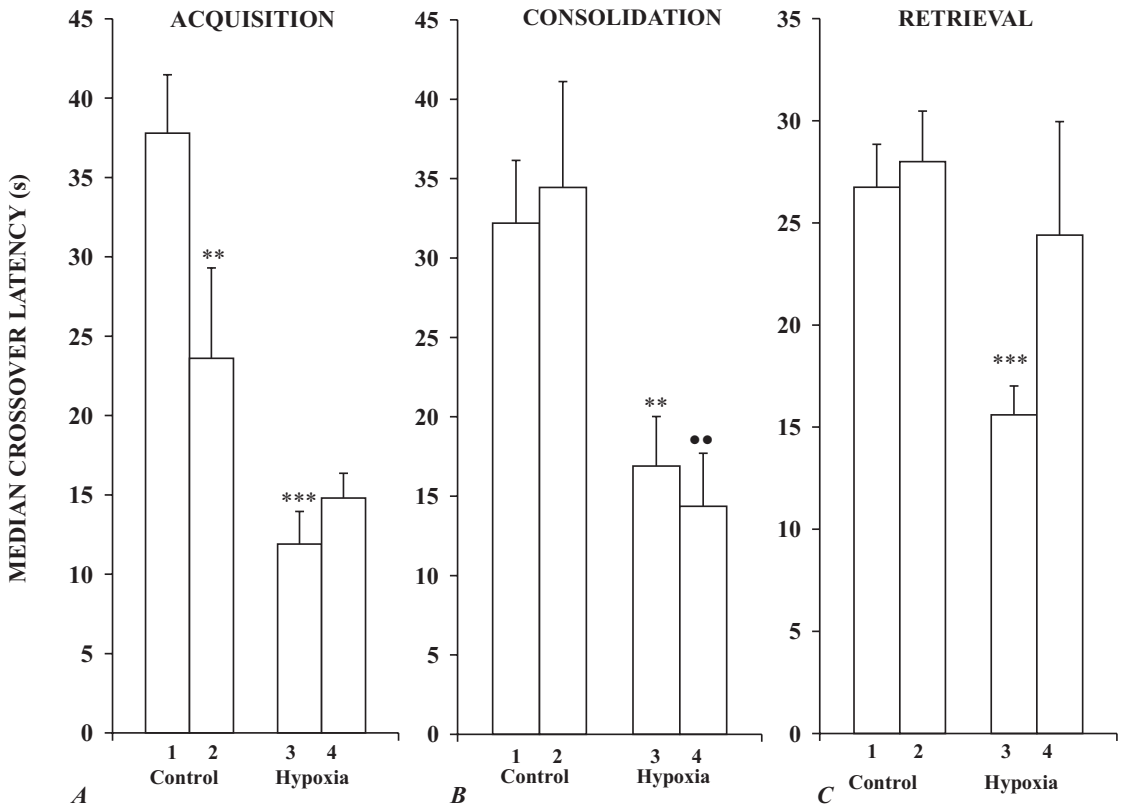

Fig. 2. The effect of saline (1), D-AP7 (2) and saline + hypoxia (3) and D-AP7 + hypoxia (4), on A) acquisition, B) consolidation, C) retrieval of passive avoidance in rats. Columns represent means ± SEM of the values obtained from 9–12 animals. A) ** p(1–2) < 0.01; *** p(1–3) < 0.001; B) ** p(1–3) < 0.01; •• p(2–4) < 0.01; C) *** p(1–3) < 0.001 (Mann-Whitney test)

The effect of D-AP7 on the activity of control and hypoxia-treated rats in the elevated plus maze (Fig. 3a, 3b)

D-AP7 in naive group of rats did not evoke significant changes in the elevated plus maze test. Hypoxia did not significantly change the time spent in closed and open arms and the number of entries into closed and open arms. D-AP7 shortened the time spent in open arms and decreased the number of entries into open arms in hypoxia-treated group of rats.

DISCUSSION

Our present study showed that the blockade of NMDA receptor by D-AP7 impaired acquisition, but did not significantly influence consolidation and retrieval in the passive avoidance situation. In hypoxia-treated groups of rats, D-AP7 did not significantly change acquisition, consolidation and retrieval in the passive avoidance response vs. hypoxia-treated group, but it impaired consolidation vs. D-AP7-treated control groups of rats.

NMDA receptor subtype plays a key role in synaptic plasticity and learning and memory processes [9]. LTP, an activity-induced rise in the efficacy of neurotransmission, has been conceived to be a physiological correlate of learning and memory [17]. Administration of competitive NMDA receptor antagonists has been shown that block induction but not maintenance of LTP [13]. Mondadori et al. [24] found that NMDA receptor antagonists could enhance or impair learning performance in animals. Certain forms of associative learning were inhibited by NMDA receptor antagonists [7]. Literature data described, similarly to our results, that NMDA receptors antagonists impaired learning acquisition, but not memory consolidation process [15, 19, 42].

In our previous and present experiments, hypoxia profoundly impaired memory processes in passive avoidance test. Hypoxia caused functional injury of the hippocampus, which is involved in particular in memory processes. During hypoxia

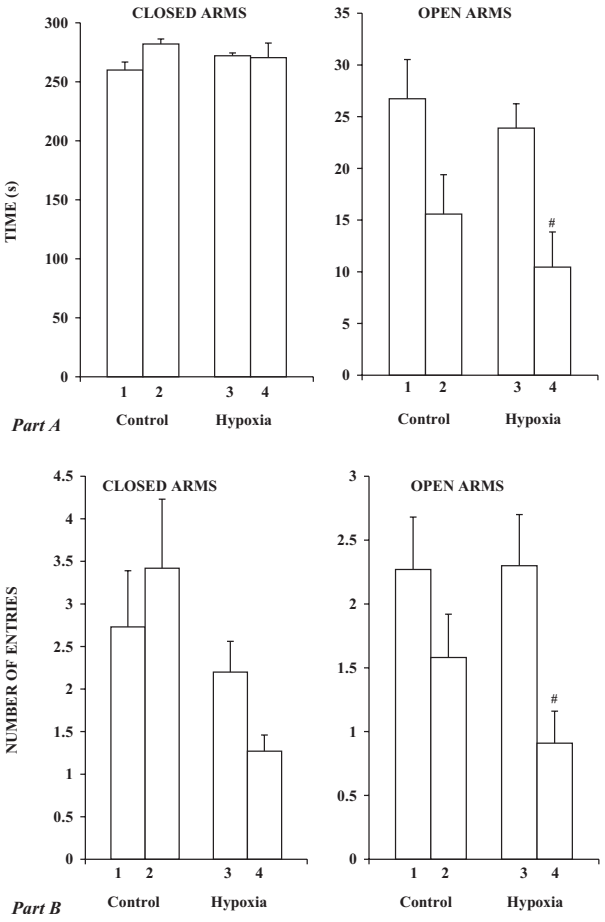

Fig. 3. The effects of saline (1), D-AP7 (2) and saline + hypoxia (3) and D-AP7 + hypoxia (4), part A) on the time spent in closed and open arms; part B) on the number of entries into closed and open arms in the elevated plus maze. Columns represent means ± SEM of the values obtained from 10–12 animals. Part A) Closed arms $F(3.40) = 1.528$; open arms $F(3.40) = 4.703$; # $p(3–4) < 0.05$; part B) Closed arms $F(3.40) = 2.467$; open arms $F(3.40) = 3.485$; # $p(3–4) < 0.05$ (ANOVA, Newman-Keuls tests)

extracellular levels of glutamate and other neurotransmitters increase [34]. Marini et al. [18] have suggested that the NMDA receptor plays a major role in mediating hypoxic-ischemic neuronal injury. The activation of NMDA receptor leads to calcium influx and activation of Ca^{2+}/calmodulin-dependent kinases. In hypoxic conditions, inhibition of calcium influx by antagonists of NMDA receptor could be neuroprotective, and blockade of EAA receptors may protect anoxic hippocampal slices [33, 39].

The consolidation process is closely connected with the hippocampus. Aitken et al. [2] reported the lack of protective effect of DL-AP7 against hypoxic damage in CA1 region of hippocampal slices. D-AP7 did not significantly change acquisition, consolidation and retrieval of passive avoidance in rats subjected to hypoxia vs. hypoxia-treated control groups of rats. Hypoxia induced memory deficits (acquisition, consolidation, retrieval) in passive avoidance test, but the blockade of NMDA receptors by D-AP7 did not prevent the impairment of these processes.

The changes in locomotor activity induced by D-AP7 might have affected the data of passive avoidance in our study. In the present experiments, the blockade of NMDA receptor by D-AP7 had no significant effects on motor activity of rats in the open field test, but we obtained tendency to enhance motility of rats. Hypoxia profoundly impaired locomotor and exploratory activity of animals. D-AP7 in hypoxia-treated group increased the number of crossed fields, rearings and bar approaches.

Some literature data showed that NMDA receptor antagonists, but especially non-competitive ones, increased locomotor activity of animals [37]. A comparison between behavioral data and dopamine (DA) release implies that the potency of the NMDA receptor antagonists to induce locomotion depends also on their potency to activate mesolimbic dopaminergic system, because uncompetitive NMDA receptor antagonists increase DA release in the nucleus accumbens [16]. The dopaminergic neurons in all areas of the brain are most sensitive to hypoxia. The results given by Miwa et al. [22] suggest that hypoxia decreases the biosynthesis of DA and the DA turnover rate was remarkably low in the whole brain. However, significant increase in extracellular DA level has been recorded in the striatal brain region in *in vivo* models of global ischemia and cerebral hypoxia [30]. There are interactions between the glutamatergic and dopaminergic systems in mechanisms of hypoxic damage [12].

Anxiety may also influence aversively motivated behavior like the passive avoidance test, which makes use mostly of aversive stimulation. It was demonstrated that D-AP7 shortened the time spent in open arms and decreased the number of entries into open arms in the elevated plus maze only in hypoxia-exposed group of rats.

Padovan et al. [29] reported that D-AP7 had no effect on any anxiety measure in naive rats. It is in agreement with our results obtained in the elevated plus maze test.

D-AP7 exhibited anxiogenic-like effect in rats subjected to hypoxia. This effect was not caused by changes in motility, because D-AP7 enhanced locomotor and exploratory activity in the open field test in this group of rats. We could also exclude the influence of this anxiogenic-like effect of D-AP7 in hypoxia-treated rats on the results in the passive avoidance test in our study.

Summarizing, hypoxia profoundly impaired the learning and memory processes in passive avoidance situation and reduced locomotor activity. D-AP7, the competitive antagonist of NMDA receptor, which by itself did not significantly affect locomotor and exploratory activity and did not produce any anxiogenic effect, impaired acquisition in the passive avoidance. In rats subjected to hypoxia, D-AP7 limited the reduction of mobility induced by hypoxia, exhibited anxiogenic-like effect and did not prevent the impairment of learning and memory processes in passive avoidance test.

In conclusion, hypoxia changed some behavioral effects of selective blockade of NMDA receptor.

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