

EFFECT OF (S)-3,5-DHPG ON LEARNING, EXPLORATORY ACTIVITY AND ANXIETY IN RATS WITH EXPERIMENTAL HYPOXIA

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We investigated the effects of (S)-3,5-DHPG, a selective agonist of group I metabotropic glutamate receptors (I mGluRs), on certain behaviors in rats after short-term hypoxia as a model of experimentally induced amnesia. The effect of (S)-3,5-DHPG administered intracerebroventricularly (*icv*) at doses of 0.01, 0.1 and 1.0 nmol was assessed using behavioral tests: the open field test, the passive avoidance response and the elevated “plus” maze test.

(S)-3,5-DHPG did not change the number of crossed fields and rearings and only at a dose of 0.01 nmol it increased the number of bar approaches in the open field test. (S)-3,5-DHPG used at all doses improved consolidation, and at doses of 0.01 and 1.0 nmol it improved retrieval in the passive avoidance test. (S)-3,5-DHPG did not produce any significant effects in control rats in the elevated “plus” maze test.

Hypoxia inhibited locomotor and exploratory activity of rats, significantly impaired consolidation and retrieval processes. We observed tendency to shortening the time spent in open arms and to decrease in the number of entries into open arms in the elevated “plus” maze in rats with underwent hypoxia.

In hypoxia-treated groups of rats, (S)-3,5-DHPG inhibited locomotor and exploratory activity in comparison with the control groups administered (S)-3,5-DHPG. Hypoxia significantly inhibited beneficial effects of (S)-3,5-DHPG on consolidation and retrieval in passive avoidance. (S)-3,5-DHPG only at the dose of 1.0 nmol used before hypoxia improved consolidation and at the dose of 0.01 nmol enhanced retrieval in comparison with saline-treated group subjected to hypoxia. (S)-3,5-DHPG only at the dose of 1.0 nmol in hypoxia-treated group shortened the time spent in closed arms and increased the number of entries into closed and open arms in the elevated “plus” maze vs saline-treated group subjected to hypoxia.

In rats subjected to hypoxia, (S)-3,5-DHPG, the agonist of I mGluRs, improved consolidation and retrieval and exhibited anxiolytic activity in dose-dependent manner.

Key words: (S)-3,5-DHPG, hypoxia, behavior, rats

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INTRODUCTION

Glutamate is the main excitatory neurotransmitter, which plays an important role in a wide variety of central nervous system (CNS) functions, ranging from memory and learning to neuronal degeneration [10, 15]. In the mammalian CNS there are two major types of the glutamate receptors: ionotropic (iGluRs) and metabotropic receptors (mGluRs) [24]. The iGluRs are intrinsic glutamate-gated ion channels for sodium and calcium cations. The mGluRs belong to the superfamily of G-protein-coupled receptors that modulate the production of intracellular second messengers [22].

The mGluRs are subdivided into three groups on the basis of their sequence similarities, signal transduction mechanisms and pharmacological specificity [10, 15]. Activation of group I mGluRs (mGluR 1 and mGluR 5) via G protein stimulates phospholipase C and phosphoinositol hydrolysis [10]. A high density of mGluR 1 and mGluR 5 was found in the hippocampus and cerebellum, i.e. in the structures, which are important for synaptic plasticity [4]. Glutamate receptors play a role in the induction of LTP (long-term potentiation) in the hippocampus in rats [27, 29]. 1S,3R-ACPD, non-selective agonist of this group significantly improved consolidation of affectively motivated memory, but failed to influence recognition memory and diminished dopaminergic transmission [37]. (S)-3,5-Dihydroxyphenylglycine [(S)-3,5-DHPG], a selective agonist of group I mGluRs, activates mGluR₁ and mGluR₅ receptors with EC₅₀ values below 10 μM. However, it should be noted that in some cell lines, the affinity of (S)-3,5-DHPG for group I mGluR is closer to 30 μM [17]. The potency (EC₅₀) of (S)-3,5-DHPG is: mGluR_{1a} = 6.6 μM, mGluR_{5a} = 2 μM, mGluR_{2, 4, 7, 8} > 1000 μM, mGluR₃ = 106 μM [10]. (S)-3,5-DHPG facilitated LTP [18], improved consolidation in dose-dependent manner and did not have an influence on retrieval in a step-through passive situation [38].

Under certain pathological conditions, including hypoxia, glutamate can become a neurotoxin and produce both immediate injury and delayed neuronal death [30]. During hypoxia, the increased synaptic release and impaired cellular reuptake of glutamate results in large increases in extracellular glutamate [5, 12, 30]. On the other hand, in our previous works, we noted that hypoxia profoundly impaired some behavioral patterns in rats [7, 8].

In the present study, we tested the effect of (S)-3,5-DHPG on learning, exploratory activity and anxiety in rats subjected to experimental hypoxia.

MATERIALS and METHODS

Animals

The study was conducted on white, male Wistar rats weighing 160–180 g. The animals were fed standard diet and housed in group cages in an air conditioned room under 12 h light/12 h dark cycle beginning at 07 h. All experiments were carried out between 8.00 h and 12.00 h.

Drug administration

(S)-3,5-DHPG, (Tocris Cookson, UK), [3] was administered into the lateral ventricle of the brain (*icv*) [14] at doses of 0.01, 0.1 and 1.0 nmol/5 μl [6, 34]. Two days before behavioral tests, a burr hole 0.5 mm in diameter was drilled in the rat's skull, 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. Ether anesthesia was used. *Icv* injection was made to a depth of 4.5 mm with a Hamilton microsyringe. After termination of each experiment, all animals were killed by decapitation, their brains were removed, and the site of injection was verified macroscopically. Animals with inappropriate injection sites were not included in the analysis.

Hypoxia induction

Hypoxia was produced by placing rats in a glass chamber flushed with a mixture of 2% O₂ in N₂ [2] till the respiratory arrest, after which they were immediately transferred to air. The hypoxia was induced 20 min before placing animals in the open field and elevated "plus" maze. In the passive avoidance situation, hypoxia was induced on the second day immediately after completion of training, or 20 min before on the third day, when we determined the effect of hypoxia on 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 cutoff time of 300 s. To determine the effect of drug treatment on retrieval, according to the protocol proposed by Matthies [19], (S)-3,5-DHPG was administered on the third day 20 min before retention test. To determine (S)-3,5-DHPG effect on consolidation, the drug was given immediately after the completion of induction of passive avoidance. Immediately after the injection of (S)-3,5-DHPG the rats were subjected to hypoxia.

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. (S)-3,5-DHPG was given 20 min before the test and then immediately the rats 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 the facilitation of exploratory behavior. The experimental procedure was similar to that described by Pellow et al. [25]. Immediately after the pretest exposure, rats were placed in the centre 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. (S)-3,5-DHPG was given 15 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 test, except for passive avoidance behavior which was assessed with Mann-Whitney ranking test. *F*-ratios, 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 (S)-3,5-DHPG on locomotor and exploratory activity of control and hypoxia-treated rats in the open field test

(S)-3,5-DHPG did not significantly change the number of crossed fields and rearings, only at the dose of 0.01 nmol it increased bar approaches. Rats subjected to hypoxia displayed significant reduction of crossings and rearings. In hypoxia-treated rats, (S)-3,5-DHPG inhibited locomotor and exploratory activity to the level of saline- and hypoxia-treated group (Fig. 1).

The effect of (S)-3,5-DHPG on consolidation of passive avoidance in control and hypoxia-treated rats

(S)-3,5-DHPG at all examined doses (0.01, 0.1 and 1.0 nmol) significantly prolonged the latency

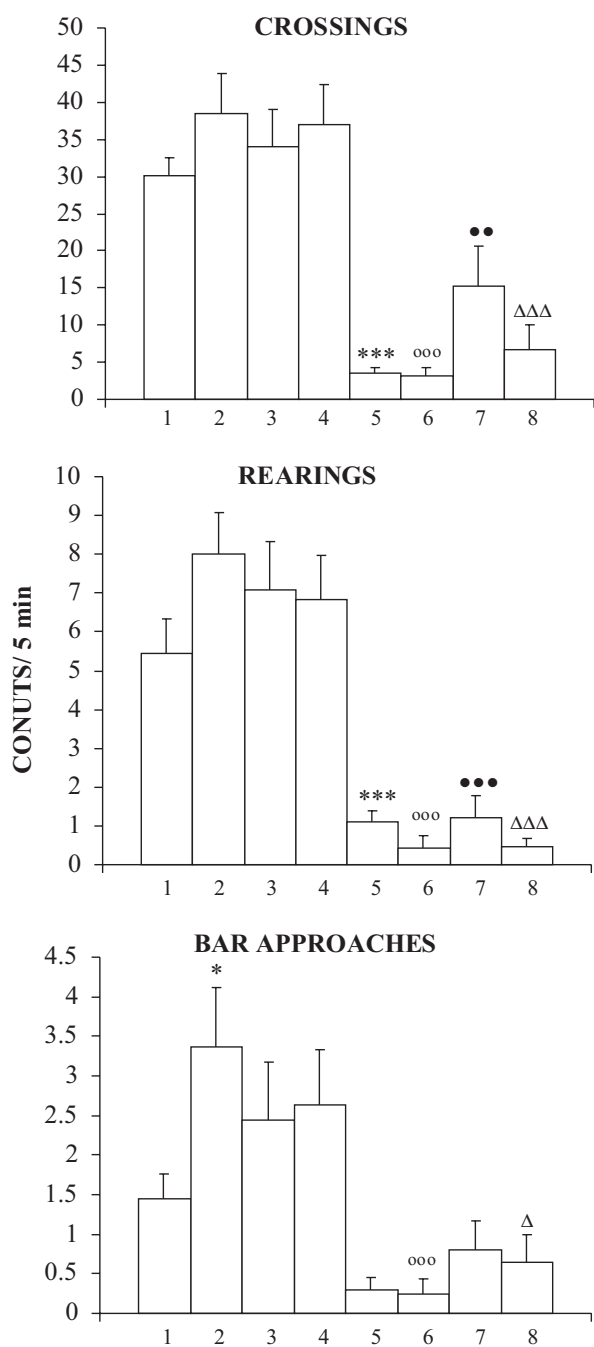


Fig. 1. The effect of (S)-3,5-DHPG used at doses of 0.01 nmol (2), 0.1 nmol (3), 1.0 nmol (4) and saline (1) in naive rats, and 0.01 nmol (6), 0.1 nmol (7), 1.0 nmol (8) and saline (5) in the rats subjected to hypoxia on the number of crossings, rearings and bar approaches in the open field test. Columns represent means \pm SEM of the values obtained from 10–12 animals. Crossings $F(7,79) = 15.333$ *** $p(1-5) < 0.001$, °°° $p(2-6) < 0.001$, ●● $p(3-7) < 0.01$, △△△ $p(4-8) < 0.001$; Rearings $F(7,79) = 16.560$ *** $p(1-5) < 0.001$, °°° $p(2-6) < 0.001$, ●● $p(3-7) < 0.001$, △△△ $p(4-8) < 0.001$; Bar approaches $F(7,79) = 5.601$ T * $p(1-2) < 0.005$, °°° $p(2-6) < 0.001$, △ $p(4-8) < 0.05$; (ANOVA and Newman-Keuls test)

of entrance to dark compartment. The time spent on the platform was shortened in rats which underwent hypoxia. 3,5-DHPG in hypoxia-treated rats shortened the latency vs 3,5-DHPG-treated control groups, but at the highest used dose it prolonged the latency in comparison with saline- and hypoxia-treated group (Tab. 1).

Table 1. The effect of (S)-3,5-DHPG in the control and hypoxia-treated group on consolidation in passive avoidance situation in rats

| Treatment | n | Re-entry latency (s) |
|--------------------------------|----|----------------------|
| Saline | 12 | 34.42 (22–55) |
| 0.01 nmol (S)-3,5-DHPG | 15 | 117.00 (21–300) * |
| 0.1 nmol (S)-3,5-DHPG | 12 | 104.25 (20–300) * |
| 1.0 nmol (S)-3,5-DHPG | 15 | 95.27 (18–300) ** |
| Saline/hypoxia | 12 | 13.00 (4–27) *** |
| 0.01 nmol (S)-3,5-DHPG/hypoxia | 12 | 16.67 (6–45) °°° |
| 0.1 nmol (S)-3,5-DHPG/hypoxia | 11 | 16.54 (7–46) ●●● |
| 1.0 nmol (S)-3,5-DHPG/hypoxia | 11 | 27.82 (6–66) # △△△ |

The rats were treated *icv* with (S)-3,5-DHPG at doses of 0.01, 0.1 and 1.0 nmol. The volume of injections was 5 μ l. Median latencies are given, with the 25–75 percentiles in parentheses. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared with saline-treated group, °°° $p < 0.001$ as compared with the group administered 0.01 nmol of (S)-3,5-DHPG, ●●● $p < 0.001$ as compared with the group injected 0.1 nmol of (S)-3,5-DHPG, △△△ $p < 0.001$ as compared with 1.0 nmol of (S)-3,5-DHPG, # $p < 0.05$ as compared with saline- and hypoxia-treated groups (Mann-Whitney test)

Table 2. The effect of (S)-3,5-DHPG in control and hypoxia-treated groups on retrieval in passive avoidance situation in rats

| Treatment | n | Re-entry latency (s) |
|--------------------------------|----|----------------------|
| Saline | 12 | 24.75 (10–44) |
| 0.01 nmol (S)-3,5-DHPG | 14 | 54.36 (11–103) ** |
| 0.1 nmol (S)-3,5-DHPG | 14 | 21.71 (5–47) |
| 1.0 nmol (S)-3,5-DHPG | 25 | 137.92 (35–300) *** |
| Saline/hypoxia | 13 | 13.00 (5–28) *** |
| 0.01 nmol (S)-3,5-DHPG/hypoxia | 14 | 23.93 (6–57) # °° |
| 0.1 nmol (S)-3,5-DHPG/hypoxia | 12 | 13.58 (4–50) |
| 1.0 nmol (S)-3,5-DHPG/hypoxia | 12 | 21.58 (7–88) △△△ |

The rats were treated *icv* with (S)-3,5-DHPG at doses of 0.01, 0.1 and 1.0 nmol. The volume of injections was 5 μ l. Median latencies are given, with the 25–75 percentiles in parentheses. ** $p < 0.01$, *** $p < 0.001$ as compared with saline-treated group, °° $p < 0.01$ as compared with the group administered 0.01 nmol of (S)-3,5-DHPG, △△△ $p < 0.001$ as compared with the group injected 1.0 nmol of (S)-3,5-DHPG, # $p < 0.05$ as compared with saline- and hypoxia-treated groups (Mann-Whitney test)

The effect of (S)-3,5-DHPG on retrieval of passive avoidance in control and hypoxia-treated rats

(S)-3,5-DHPG used at doses of 0.01 and 1.0 nmol significantly prolonged the time of entering the dark compartment. Hypoxia significantly shortened the latency in rats. (S)-3,5-DHPG given at doses of 0.01 and 1.0 nmol significantly shortened the time spent on the platform in rats which underwent hypoxia in comparison with rats not submitted to hypoxia. In hypoxia-treated rats (S)-3,5-DHPG only at the dose of 0.01 nmol prolonged the latency (Tab. 2).

The effect of (S)-3,5-DHPG on activity of control and hypoxia-treated rats in elevated “plus” maze

(S)-3,5-DHPG did not produce any effects in the elevated plus maze. We observed not significant tendency to shortening the time spent in open arms (Fig. 2B) and to decrease in the number of entries into open arms (Fig. 3B) in rats subjected to hypoxia. In hypoxia-treated group (S)-3,5-DHPG used at dose of 1.0 nmol shortened the time spent in closed arms (Fig. 2A) and increased the number of entries into closed and open arms (Fig. 3A, B)

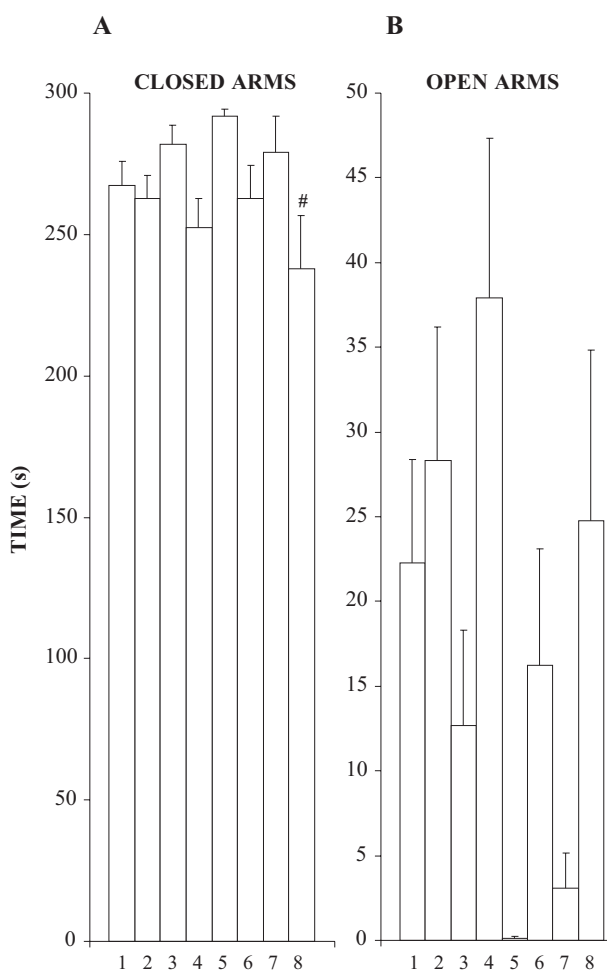


Fig. 2. The effect of (S)-3,5-DHPG at dose 0.01 nmol (2); 0.1 nmol (3); 1.0 nmol (4) and saline (1) in naive rats, and 0.01 nmol (6), 0.1 nmol (7), 1.0 nmol (8) and saline (5) in hypoxia-treated rats on the time spent in the closed (A) or open arms (B) of elevated “plus” maze. Columns represent means \pm SEM of the values obtained from 9–13 animals A) Closed arms $F(7.77) = 2.467$ # $p(5-8) < 0.05$; B) Open arms $F(7.77) = 3.157$ (ANOVA and Newman-Keuls test)

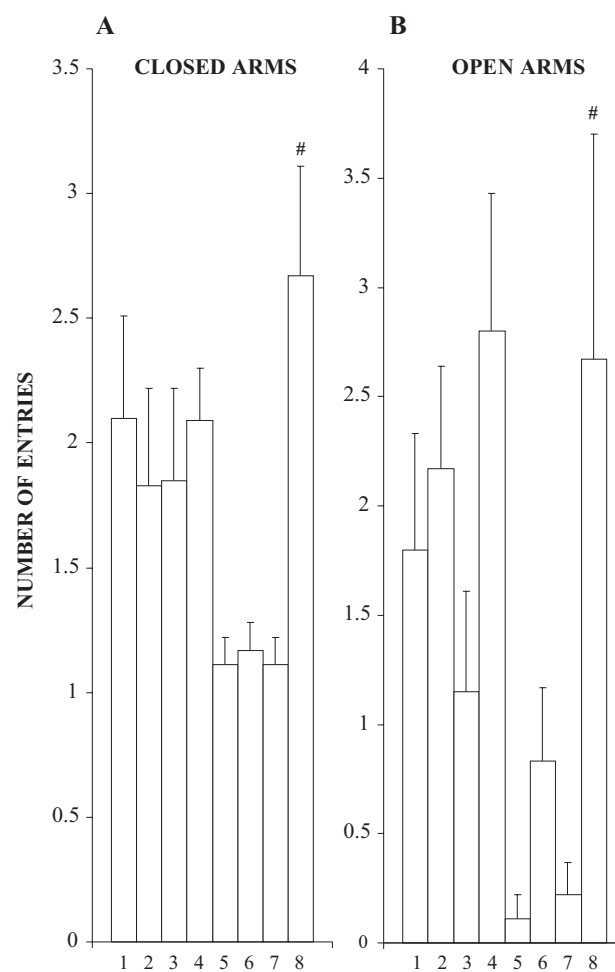


Fig. 3. The effect of (S)-3,5-DHPG at the doses 0.01 nmol (2), 0.1 nmol (3), 1.0 nmol (4), and saline (1) in naive rats, and 0.01 nmol (6), 0.1 nmol (7), 1.0 nmol (8) and saline (5) in hypoxia-treated rats on the number of entries into closed (A) and open arms (B) of elevated “plus” maze. Columns represent means \pm SEM of values obtained from 9–13 animals. A) Closed arms $F(7.77) = 3.035$ # $p(5-8) < 0.05$ B) Open arms $F(7.77) = 3.688$ # $p(5-8) < 0.05$ (ANOVA and Newman-Keuls test)

in comparison with the saline- and hypoxia-treated group.

DISCUSSION

In our present study, hypoxia inhibited locomotor and exploratory activity of rats in control and (S)-3,5-DHPG-treated groups.

Hypoxia disturbs synthesis and release of many neurotransmitters. Ischemia/hypoxia simulated *in vitro* provokes a Ca^{2+} -independent liberation of glutamate, aspartate, GABA, glycine and taurine. During complete ischemia/hypoxia, the concentration of extracellular glutamate increases 100-fold [5]. Moreover, hypoxia decreases the biosynthesis and turnover rate of noradrenaline and dopamine (DA), and dopaminergic neurons are very sensitive to hypoxia in all regions of the brain [21]. In our experiment, rats after hypoxia exhibited significant decrease in crossings and rearings. The observed inhibition of locomotion in hypoxia-treated groups of rats is probably caused by the influence of hypoxia on dopaminergic system.

mGluRs are involved in the generation of DA-dependent locomotor behaviors [33, 35], and they also depress monosynaptic excitation of the rat spinal motoneurons [16]. The low dose of (S)-3,5-DHPG (20 nmol) did not produce any detectable change in locomotion while at the dose of 40 nmol, (S)-3,5-DHPG started to increase locomotion [36]. In the present study, (S)-3,5-DHPG used *icv* at doses of 0.01, 0.1 and 1.0 nmol did not change locomotor activity in the open field test. (S)-3,5-DHPG in hypoxia-treated groups of rats inhibited locomotor and exploratory activity in comparison with (S)-3,5-DHPG-treated control groups. This effect probably consists in hypoxia-dominated influence on dopaminergic system.

Our present study showed that (S)-3,5-DHPG improved consolidation (at all doses) and retrieval (at doses of 0.01 and 1.0 nmol) in the passive avoidance situation. Hypoxia significantly impaired consolidation and retrieval, and diminished beneficial effects of (S)-3,5-DHPG on these processes, but (S)-3,5-DHPG only at the dose of 1.0 nmol improved consolidation and at the dose of 0.01 nmol it enhanced retrieval in hypoxia-treated groups.

(S)-3,5-DHPG influenced learning and memory [9, 38]. Some data showed a potential role of mGluR 1 and mGluR 5 in the hippocampus-dependent form of learning, like passive avoidance [23].

Riedel and Reymann [27] demonstrated that the activation of mGluRs is essential for induction and maintenance of LTP in the hippocampus. The agonists of group I mGluRs may selectively increase the signal-to-noise ratio and thus cause the memory facilitation [28].

According to some studies, the group I mGluRs are involved in nociceptive processes [13]. The nociceptive property of (S)-3,5-DHPG could not interfere with the results obtained in a passive avoidance situation, because rats received the compound just after the learning trial (after the footshock). In learning situation, the activation of group I mGluRs by (S)-3,5-DHPG is limited to a time window during and shortly after learning acquisition.

The present and earlier studies demonstrated that hypoxia impaired the memory processes [7, 8]. Hypoxic episodes are likely to underlie neuronal damage. Hypoxia induces the hypoxic-inducible factor-1 (HIF-1), a transcriptional activator that binds to many genes and plays a key role in the regulation of the genes involved in apoptosis [32]. Free radicals and high concentration of excitatory amino acids are neurotoxic and contribute to the neuronal death during hypoxia [11]. Among the various brain areas, the hippocampus is particularly vulnerable to such ischemic injury and subsequent cell death [26]. The hippocampus plays an important role in memory, especially in consolidation process.

The enhancement of consolidation and retrieval in passive avoidance test in rats subjected to hypoxia and pretreated with (S)-3,5-DHPG is beneficial from clinical point of view. In the presence of (S)-3,5-DHPG, the function of L-type of calcium channels in hypoxia conditions was impaired [20]. Schröder et al. [31] have suggested that (S)-3,5-DHPG is protective when present prior to the onset of the hypoxic event. On the other hand, on the basis of our experiments we can exclude the influence of rats' mobility on consolidation and retrieval processes because we observed locomotor activity inhibition in the open field test in (S)-3,5-DHPG- and hypoxia-treated groups of rats. Anxiety also could bias the results in the passive avoidance test, which uses aversive stimulation.

In our study, we observed only tendency to shortening the time spent in open arms in elevated "plus" and to decrease the number of entries into open arms in rats having undergone hypoxia maze. In hypoxia-treated group, (S)-3,5-DHPG used at the dose of 1.0 nmol shortened the time spent in

closed arms and increased the number of entries into closed and open arms in comparison with the saline- and hypoxia-treated group.

The results obtained in elevated "plus" maze test cannot be connected with beneficial activity of (S)-3,5-DHPG on consolidation and retrieval in the passive avoidance in the control and hypoxia-treated groups. Rats in (S)-3,5-DHPG-treated groups did not exhibit anxiogenic activity.

In conclusion, hypoxia impaired consolidation, retrieval processes in the passive avoidance situation and reduced locomotor and exploratory activity. (S)-3,5-DHPG improved the memory processes and it did not significantly change activity of rats in the open field and in the elevated "plus" maze. Hypoxia in (S)-3,5-DHPG-treated rats inhibited locomotor and exploratory activity, consolidation and retrieval processes in comparison with (S)-3,5-DHPG-treated control groups. However, (S)-3,5-DHPG only at the dose of 1.0 nmol improved consolidation and at the dose of 0.01 nmol it enhanced retrieval in hypoxia-treated groups. Because (S)-3,5-DHPG was given before induction of hypoxia, we may suggest that this agonist of mGluRs had preventive effects on hypoxia-induced memory impairment.

Summarizing, in spite of hypoxia, (S)-3,5-DHPG positively influenced the memory processes in the dose-dependent manner.

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