

INFLUENCE OF AIDA ON CERTAIN BEHAVIORS IN RATS SUBJECTED TO EXPERIMENTAL HYPOXIA

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Influence of AIDA on certain behaviors in rats subjected to experimental hypoxia. A. NADLEWSKA, H. CAR, R.J. WIŚNIEWSKA, K. WIŚNIEWSKI. Pol. J. Pharmacol., 2003, 55, 171–180.

The influence of the blockade of group I metabotropic glutamate receptors (mGluRs) by AIDA [(RS)-1-aminoindan-1,5-dicarboxylic acid] on some behavioral effects was tested in control groups of rats and in rats that underwent short-term hypoxia. We used the following methods: the open field test, the passive avoidance test and the elevated “plus” maze test.

In rats without hypoxia, AIDA (100 nmol *icv*) decreased the number of crossings in the open field test, impaired acquisition, improved consolidation and did not influence retrieval in the passive avoidance situation and was ineffective in the elevated “plus” maze.

Short-term hypoxia (2% O₂, 98% N₂), as a model of experimentally induced amnesia, significantly inhibited locomotor and exploratory activity and profoundly impaired acquisition, consolidation and retrieval processes and did not exhibit proanxiogenic or anxiolytic effect in elevated “plus” maze.

AIDA (100 nmol *icv*) used before hypoxia significantly improved consolidation and retrieval processes, but had no effect on acquisition and did not significantly influence all parameters of the elevated “plus” maze test.

The obtained results suggest that AIDA, the selective antagonist of group I mGluRs, had beneficial effects on consolidation and retrieval of passive avoidance in rats undergoing hypoxia.

Key words: *AIDA, passive avoidance, locomotion, elevated “plus” maze, hypoxia, rats*

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INTRODUCTION

Glutamate is an endogenous excitatory neurotransmitter that is utilized by many neurons in the mammalian central and peripheral nervous system. Glutamate through its many receptors mediates most of the excitatory neurotransmission in the central nervous system (CNS) [31]. Two principal types of glutamate receptors have been identified: ligand-gated ion channels, i.e. NMDA, AMPA and kainate receptors (iGluRs), which mediate fast excitatory transmission, and G-protein-coupled receptors, i.e. metabotropic glutamate receptors (mGluRs), which indirectly regulate electric signaling and activate various second messenger cascades [22, 46]. Eight different subtypes of mGluRs are subclassified into three groups on the basis of their amino acid sequence similarities and the second messenger systems that they act on. Group I mGluRs (mGluR1 and mGluR5) are linked *via* G protein to phospholipase C and phosphoinositol hydrolysis [17].

Glutamatergic neurotransmission is involved in biochemical events underlying learning and memory processing [5]. mGluRs are critically involved in synaptic plasticity of various brain structures, and seem to have an essential role in some learning and memory processes [42].

AIDA (1-aminoindan-1,5-dicarboxylic acid) is a potent and specific antagonist of group I mGluRs [28]. Christoffersen et al. [15, 16] reported that the blockade of group I mGluRs might enhance short-term memory under certain learning conditions in rats.

Hypoxia induces deficits of memory [12, 30]. A major mechanism of neuronal damage during ischemia is the massive release of endogenous glutamate and over-stimulation of excitatory amino acids receptors in certain brain areas which leads to neuronal death. Excitotoxic neuronal injury appears to be mediated through stimulation of iGluRs and mGluRs [8, 33]. Group I mGluRs probably play a role in oxygen glucose deprivation (OGD) and ischemic brain damage. Antagonists of group I mGluRs proved to be neuroprotective [9, 10, 36].

The aim of our study was to investigate the influence of AIDA on certain behaviors, such as learning, locomotor and exploratory activity, anxiety, in rats subjected to hypoxia as a model of experimentally induced amnesia.

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 h and 12.00 h. Each animal was used only once and the same rat was not used in different tests.

Surgery

The rats were anesthetized with chloral hydrate at a dose of 0.4 g/kg *ip*. A round piece of skin 7 mm in diameter was cut off the rat's head and the underlying skull surface was cleaned off the soft tissue. A burr hole of 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 [21]. After 48 h of recovery, the wound was completely dry and the animal behaved normally. The intracerebroventricular (*icv*) injections of AIDA were made freehand into the lateral cerebral ventricles with a 10 µl Hamilton syringe, using a removable KF 730 needle 4.5 mm long. This procedure allowed lowering the tip of the needle about 0.5 mm below the ceiling of the lateral cerebral ventricle. It was relatively nontraumatic as the animal, gently fixed in the left hand of the experimenter, was usually quiet and no vocalization occurred. The injection volume was 5 µl administered for over 1 min. After termination of each experiment, all animals were sacrificed, their brains were removed, and the sites of injection were verified macroscopically after brain sectioning. Animals with inappropriate injection sites were not considered for analysis.

Drugs

(RS)-1-Aminoindan-1,5-dicarboxylic acid (AIDA, Tocris Cookson, UK) was administered only once into the lateral ventricle of the brain (*icv*) [21] at a dose of 100 nmol per single rat in the volume of 5 µl [15]. 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 mixture of 2% O₂ in 98% N₂ [3] till the respiratory arrest (about 3 min), after which they were immediately transferred to air. The hypoxia was induced 15 min before placing animals in the open field test and elevated “plus” maze. In the passive avoidance situation hypoxia was induced on the second day 15 min before training, or immediately after completion of training, or 15 min before it on the third day, when we determined the effect of hypoxia on acquisition, consolidation or retrieval, respectively. An animal was submitted to hypoxia only once.

Behavioral testing

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, the rats were placed on an illuminated platform, allowed to enter the dark compartment and then 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. At the end of the second trial, when a rat entered the dark compartment it received an inescapable footshock (0.25 mA, 3 s) delivered through the grill floor of the dark compartment (learning trial). Retention of passive avoidance was checked 24 h later by measuring the latency to re-enter the dark compartment up to a maximum of 300 s. To determine the effect of drug treatment on retrieval, according to the protocol proposed by Matthies [25], AIDA was administered on third day 15 min before retention test. To determine AIDA effect on consolidation, the drug was given on the second day immediately after completion of induction of passive avoidance or 15 min before it to determine AIDA effect on acquisition. Immediately after the injection of AIDA the rats were subjected to hypoxia.

Locomotor and exploratory activity

The open field test was used to estimate the locomotor (crossings) and exploratory (rearings, bar approaches) activity of rats. The apparatus consisted of a square 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 (designed as objects of possible interest), 20 cm high, were located at four different line crossings in the central area of the floor. A single rat was placed in the centre of the floor and allowed 1 min of adaptation. Subsequently, crossings, rearings, and bar approaches were counted manually for 5 min. The crossings of the square were counted when the animal crossed the line with all four paws and the bar approaches were considered when the rat directed its head toward the bar, approached and touched it with its nose. AIDA was given 15 min before the test and then immediately the rats underwent hypoxia.

Elevated “plus” maze

The maze (constructed of grey coloured wooden planks) consisted of two open arms, 50 cm (length) × 10 cm (width), and two enclosed arms, 50 cm (length) × 10 cm (width) × 40 cm (height), covered with a removable lid, so that the open or closed arms were opposite to each other. The maze was elevated to a height of 50 cm from the floor. Ten 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. [38]. 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 entering with all four feet into one arm. An increase in open arm entries and increase in time spent in the open arms is indicative of potential anxiolytic activity, as rats naturally prefer the closed arms. AIDA 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 Newman-Keuls test, 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 Ethics Committee of Medical Academy in Białystok.

RESULTS

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

AIDA decreased the number of crossed fields and displayed no significant tendency to reduction of rearings and bar approaches. Rats subjected to hypoxia

showed profoundly impaired locomotor and exploratory activity, this effect did not change after AIDA.

The effect of AIDA on acquisition of passive avoidance in control and hypoxia-treated rats (Fig. 2A)

AIDA significantly shortened the latency in rats. Hypoxia also shortened the time spent on the luminated platform. AIDA in hypoxia-treated rats did not significantly change the latency.

The effect of AIDA on consolidation of passive avoidance in control and hypoxia-treated rats (Fig. 2B)

The latency was shortened after hypoxia vs control group of rats. AIDA significantly prolonged

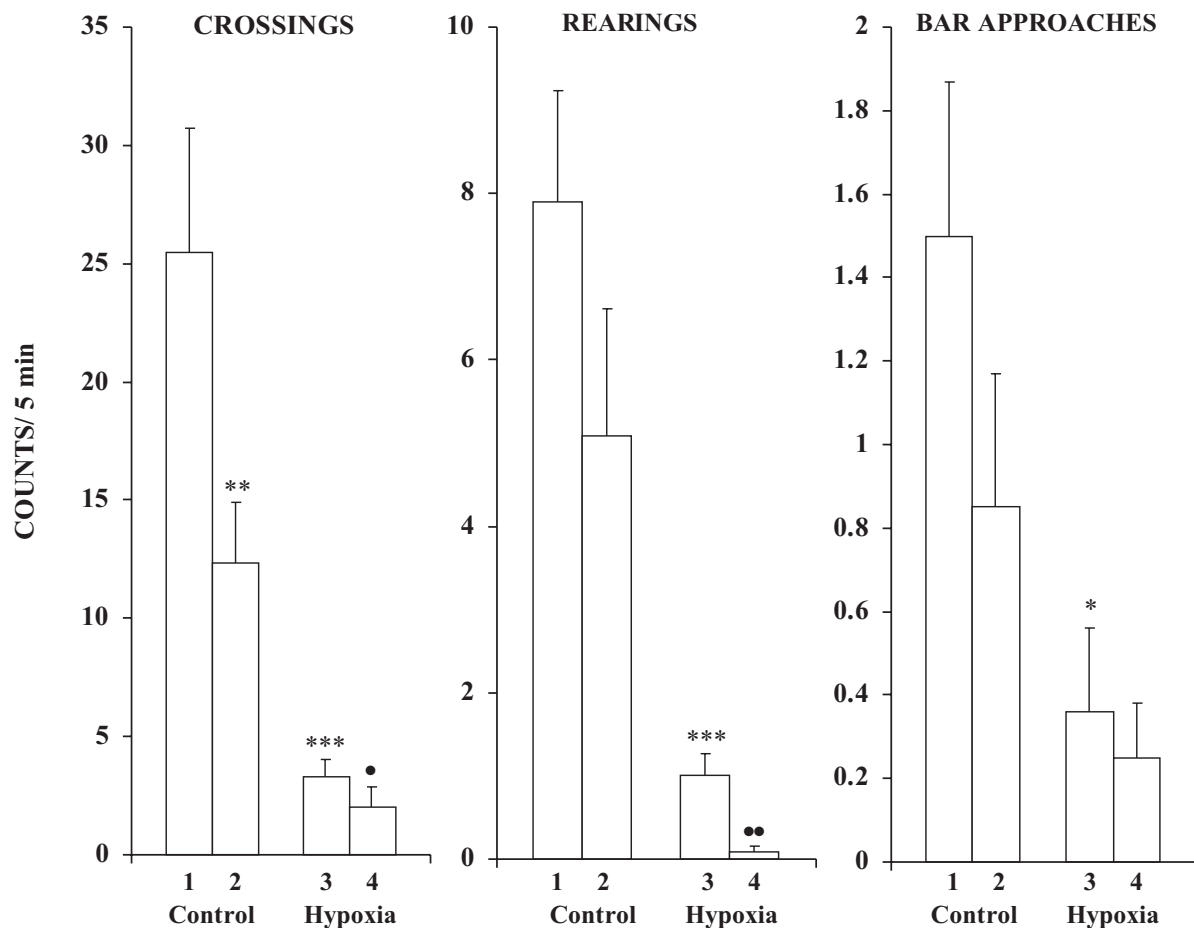


Fig. 1. The effect of AIDA on number of crossings, rearings and bar approaches in the open field in control (groups 1–2), and hypoxia-treated rats (groups 3–4). Columns represent means \pm SEM of the values obtained from 10–12 animals. 1) saline (5 μ l 0.9% NaCl *icv*); 2) AIDA (100 nmol *icv*); 3) saline (5 μ l 0.9% NaCl *icv*) and hypoxia; 4) AIDA (100 nmol *icv*) and hypoxia. Crossings $F(3,42) = 14.156$; ** $p(1-2) < 0.01$; *** $p(1-3) < 0.001$; • $p(2-4) < 0.05$. Rearings $F(3,42) = 11.289$; *** $p(1-3) < 0.001$; •• $p(2-4) < 0.01$. Bar approaches $F(3,42) = 4.137$; * $p(1-3) < 0.05$ (ANOVA, Newman-Keuls tests)

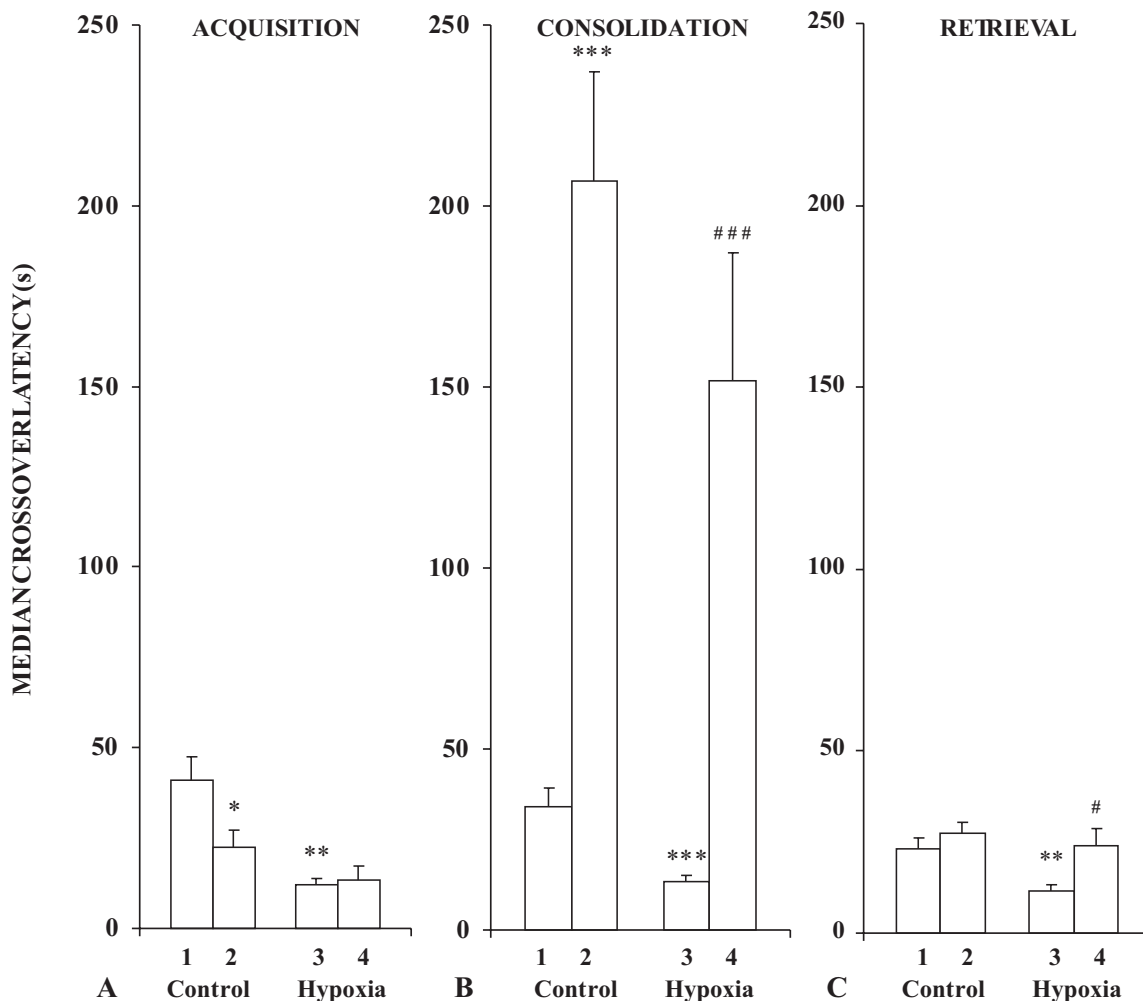


Fig. 2. The effect of saline (1), AIDA (2) and saline + hypoxia (3) and AIDA + hypoxia (4), on A) acquisition, B) consolidation, C) retrieval of passive avoidance in rats. Columns represent means \pm SEM of the values obtained from 10–15 animals. A) * $p(1-2) < 0.05$; ** $p(1-3) < 0.01$; B) *** $p(1-2,3) < 0.001$; ### $p(3-4) < 0.001$; C) ** $p(1-3) < 0.01$; # $p(3-4) < 0.05$ (Mann-Whitney test)

the time to entrance to dark compartment in groups of rats submitted and not submitted to hypoxia.

The effect of AIDA on retrieval of passive avoidance in control and hypoxia-treated rats (Fig. 2C)

AIDA did not significantly change the time spent on the platform. Hypoxia shortened the latency in rats, but this effect was reversed by administration of AIDA.

The effect of AIDA on the activity of control and hypoxia-treated rats in the elevated “plus” maze (Fig. 3 part A, part B)

AIDA did not significantly change the time spent in the open and closed arms (part A) and the

number of entries into the open and closed arms (part B) in rats submitted and not submitted to hypoxia. Hypoxia did not significantly influence all parameters of the elevated “plus” maze.

DISCUSSION

In our present experiments, we observed that AIDA, the selective antagonist of group I mGluRs, administered *icv* at the dose of 100 nmol improved consolidation, impaired acquisition and did not influence retrieval in passive avoidance situation. Hypoxia significantly impaired memory process. AIDA attenuated consolidation and retrieval deficit induced by hypoxia and had no effect on hypoxia-evoked impairment of acquisition in passive avoidance test.

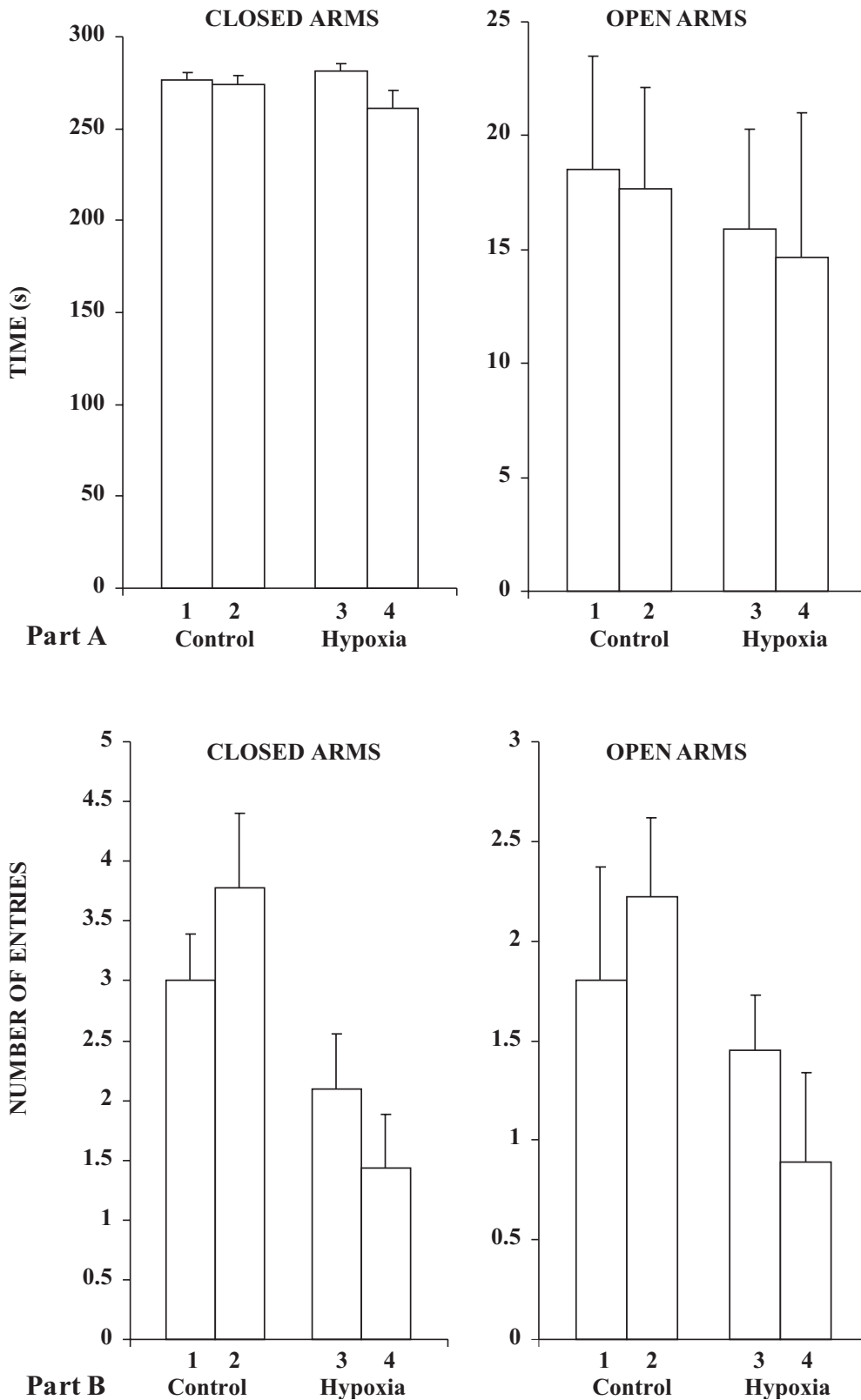


Fig. 3. The effects of saline (1), AIDA (2) and saline + hypoxia (3) and AIDA + hypoxia (4) (part A) on the time spent in closed and open arms, and (part B) on the number of entries into closed and open arms in the elevated "plus" maze. Columns represent means \pm SEM of the values obtained from 9–11 animals. Part A) Closed arms $F(3.35) = 2.099$; open arms $F(3.35) = 0.113$; part B) Closed arms $F(3.35) = 4.285$; open arms $F(3.35) = 1.561$ (ANOVA, Newman-Keuls tests)

Hölscher et al. [22] reported that the blockade of group I mGluRs prevented induction of LTP and learning in various experimental models. According to some data, mGluR5 knockout mice exhibited the inhibition of LTP in CA1 and dentate gyrus, and they showed impairment of acquisition [23]. In our study, we also observed inhibition of acquisition in passive avoidance situation after injection of AIDA. Nielsen et al. [34] suggested that learning and memory deficits could be evoked by selective blockade of group I mGluRs in CA1 region of the hippocampus. However, Bordi and Ugolini [7] demonstrated that antagonists of the mGluRs did not prevent induction of LTP in the dentate gyrus of rats. Christoffersen et al. [15, 16] described that antagonists of group I mGluRs could facilitate short-term memory. AIDA improved short-term memory, but impaired long-term spatial learning in rats [15, 16].

In our experiments, blockade of group I mGluRs by AIDA had different influence on various phases of memory formation in passive avoidance test. In previous studies, we demonstrated that the administration of antagonist of group I mGluRs AIDA or the selective antagonist of mGluR5 MPEP improved consolidation process in passive avoidance situation [11, 30]. It suggests that the blockade of neurotransmission induced by group I mGluRs had beneficial effect on consolidation process.

In our study, short-term hypoxia induced by 2% O₂ and 98% N₂ impaired acquisition, consolidation and retrieval in passive avoidance situation. It is unclear so far why hypoxia inhibits memory processes. Hypoxia disturbs homeostasis between neurotransmitter systems [4]. An increase in extracellular level of glutamate was observed during hypoxia [43, 45]. Neurotoxic effect induced by the high extracellular concentration of excitatory amino acids and free radicals leads to neuronal death [19]. Among various regions of the brain, the hippocampus is particularly sensitive to ischemia and hypoxia. The hippocampus plays a very important role in memory formation, but especially in consolidation process [40, 44].

Under *in vivo* conditions, hypoxia or depletion of substrates for oxidative phosphorylation led to marked structural alterations in CNS in rats [40]. As a consequence of the energetic deficit, neurons of several brain regions exhibited depolarizing and/or hyperpolarizing responses to hypoxia [39].

Bolaños et Almeida [6] reported about the role of *NO generated during brain hypoxia in memory loss. In *in vivo* rat model aiming at quantifying the number of errors committed in a working memory task, inhibition of NOS activity prevented the increase in errors caused by 5 min of ischemia [35].

In experimental hypoxia, the blockade of group I mGluRs by AIDA reversed consolidation and retrieval impairment, but did not influence acquisition deficit in the passive avoidance situation.

It has been shown that antagonists of group I mGluRs are neuroprotective under *in vitro* and *in vivo* conditions [29, 32, 41, 47]. AIDA reduced acute neuronal degeneration and behavioral deficits after traumatic brain injury in rats [24]. AIDA significantly reduced neuronal cell death in mixed neocortical cell and organotypic hippocampal cultures subjected to OGD. Also *icv* administration of AIDA had neuroprotective effect on transient cerebral ischemia in gerbils [20, 36]. Moroni et al. [27] proved that AIDA inhibited synaptic release of glutamate mediated by presynaptic mGluR1 in the rat cortex. It confirmed that group I mGluRs were not always activated under basic conditions and they increased transmitter output under excitotoxic conditions.

The nociceptive property could influence the results obtained in passive avoidance test. mGluRs, especially these of group I, play an important role in modulation of nociception [37]. It has been shown that antagonists of group I mGluRs reduce electrophysiological responses evoked by nociceptive stimuli in the spinal cord and in the thalamus [13]. Administration of AIDA caused only mild analgesia [28]. This effect can be taken into consideration mainly in estimation of the effect of AIDA on acquisition. Analgetic property of AIDA could not interfere with consolidation and retrieval results, because rats received the compound just after the learning trial, so during the footshock the animals were free of the influence of the drug.

The changes in locomotor activity induced by AIDA may affect the data on passive avoidance in our study. AIDA significantly decreased the number of crossing in the open field. Hypoxia inhibited motility of rats in control and AIDA-treated groups. Some literature data described that mGluR1 knockout mice exhibited motor deficits and impairment of movement-dependent learning [2, 18]. Moroni et al. [28] indicated that AIDA-treated animals had a tendency to stay immobile in the centre of the

open field. They had difficulties in the initiation of movement, and probably it could be a cause of decreased number of crossings in rats treated in AIDA.

The observed locomotor activity inhibition in groups of rats subjected to experimental hypoxia might be in connection with dopaminergic system. Miwa et al. [26] suggested that dopaminergic neurons are very sensitive to hypoxia in all areas of the brain. Hypoxia inhibits the biosynthesis and the turnover rate of dopamine in the CNS.

The inhibition of locomotor activity induced by hypoxia in AIDA-treated group of rats could influence the retrieval improvement in passive avoidance test, because the time of AIDA injection and hypoxia induction were the same, as in open field in parallel group.

Anxiety could bias the results in passive avoidance test, which uses aversive stimulation. The ligands inhibiting glutaminergic transmission, especially antagonists of group I mGluRs, possess the anxiolytic properties [14]. We investigated the anxiolytic potential of AIDA in rats subjected to hypoxia in the elevated "plus" maze. We did not obtain any significant changes in the time spent in the open or closed arms or the number of entries into open or closed arms in AIDA-treated and hypoxia-exposed groups of rats. We could exclude anxiety influence on our results on passive avoidance.

In summary, hypoxia profoundly impaired acquisition, consolidation and retrieval in passive avoidance paradigm and it reduced the locomotor and exploratory activity. AIDA impaired acquisition and improved consolidation in the passive avoidance test. In rats exposed to hypoxia, this antagonist of group I mGluRs exhibited the beneficial activity on consolidation and retrieval, but did not influence acquisition deficit in the passive avoidance situation.

In conclusion, *icv* administration of AIDA can prevent hypoxia-induced consolidation and retrieval impairment of passive avoidance in rats.

Acknowledgment. This work was supported by grant No. 3-10689 from the State Committee for Scientific Research, Warszawa, Poland.

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Received: August 27, 2002; in revised form: March 10, 2003.