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Effects of baclofen and L-AP4 in passive avoidance test in rats after hypoxia-induced amnesia

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Abstract:
Hypoxia-induced cognitive deficits are mainly due to disturbances of the balance between the GABAergic and glutamatergic systems. Acquisition, consolidation and retention impairment in passive avoidance test, hypolocomotion in the open field test, an anxiogenic-like effect in the elevated plus-maze test and hypothermia were observed in rats subjected to hypoxia. Drugs which reduce glutamate release may possess neuroprotective activity. Both, agonists of $GABA_B$ (baclofen) and group III mGlu receptors (L-AP4) influence the release of glutamate.
We studied the behavioral effects of baclofen on hypoxia-induced amnesia and the role of L-AP4 in these processes. Baclofen impaired acquisition, produced an anxiogenic-like effect and lowered body temperature but reduced the hypoxia-induced deficit of acquisition and consolidation of conditioned avoidance, diminished the anxiogenic-like effect, and reduced the motor inhibition produced by hypoxia. L-AP4 improved the acquisition, consolidation and retrieval processes as well as the hypoxia-induced consolidation deficit in the passive avoidance test. Co-administration of baclofen with L-AP4 improved consolidation and enhanced the baclofen activity *vs.* the respective group without hypoxia. In a group of rats that had undergone hypoxia, joint administration of baclofen and L-AP4 improved retrieval as well as enhanced the effect of baclofen and L-AP4 *vs.* their respective group without hypoxia. The agonist of group III mGluRs did not change locomotor activity but diminished baclofen-induced motility in rats without hypoxia. L-AP4 given alone or with baclofen produced an anxiogenic-like effect in rats without hypoxia but produced an anxiolytic-like effect in those that had undergone hypoxia. L-AP4 did not influence the activity of baclofen in the elevated plus-maze test. L-AP4 given alone or with baclofen did not change body temperature.
It is concluded that baclofen and L-AP4 may cooperate in the consolidation process in rats without hypoxia and in retrieval of passive avoidance in animals that had undergone hypoxia. The observed interaction is probably the result of activation of the presynaptic receptors which influence glutamate and GABA release.

Key words:
hypoxia, baclofen, L-AP4, passive avoidance, open field, elevated plus-maze, rat

Introduction

Hypoxia impairs learning and memory and several studies have demonstrated that ischemic animals display impairment in performance of the passive avoidance task [5, 6, 7, 9, 28, 29]. The glutamate excitotoxicity, disturbances in many neurotransmitter systems, especially disruption of the balance between the GABAergic inhibitory and glutamatergic excitatory systems, are probably the mechanisms underlying such neurocognitive deficits. The presynaptic release of glutamate is an important initiating event in neuronal injury during and after hypoxia. Drugs which can inhibit spontaneous glutamate release may prevent the hypoxia-induced excitotoxicity of neurons [46, 47] and probably may diminish the learning and memory deficits produced by hypoxia. Both presynaptic $GABA_B$ and metabotropic glutamate (mGlu) receptors have

potential inhibitory effect during hypoxia [44]. The activation of presynaptic $GABA_B$ receptors by an agonist, baclofen, inhibits the release of GABA and glutamate in the GABAergic and glutamatergic synapses [17, 22]. Our previous study [9] indicated that baclofen had beneficial action on consolidation of conditioned avoidance in amnesia induced by hypoxia. On the other hand, the mGluR-induced modulation of GABAergic synaptic transmission has been implicated in neuronal damage [4]. In accordance with this hypothesis, we reported that group I mGluRs took part in the behavioral effects of baclofen in normoxic rats [8] and attenuated hypoxia-induced deficits of learning and memory [35, 36].

Group III mGlu receptors 4, 6, 7, and 8 are negatively coupled to the cAMP/PKA signal transduction pathway [42], have both pre- and post-synaptic localization in various neuronal glutamatergic and GABAergic pathways [31, 32], and have been found to contribute to presynaptic regulation of glutamate (as autoreceptors) and GABA transmission (as heteroreceptors) [42]. Under hypoxia/ischemia conditions, these receptors are intensively activated [3].

The beneficial behavioral activity of the selective agonist of group III mGluRs, L-2-amino-4-phosphonobutyric acid (L-AP4) [24] in normoxic rats [12, 49] and the reduction of the consolidation deficit produced by hypoxia [10] indicate that group III mGluRs are necessary for learning and memory.

In the present study, we tested the possible contribution of L-AP4 to the behavioral effects of baclofen on acquisition, consolidation and retrieval processes in the passive avoidance test in rats after hypoxia which was used as a model of amnesia. The reduced anxiety and changed locomotor activity are expected to cause impairment of passive avoidance, therefore, we measured locomotor activity in the open field test and used the elevated plus-maze test to determine the drug effects on anxiety. Additionally, we measured body temperature, because hypothermia is an indicator of the activity of baclofen and has neuroprotective effect in hypoxia.

Materials and Methods

Animals

Female Wistar rats of laboratory strain, weighing 160–180 g (3 months of age), were used. The animals were fed a standard diet and housed in plastic cages (43 × 33 × 25.5 cm), one animal per cage, in an air-conditioned and temperature-controlled (22 ± 2°C) room under a 12 h light/dark cycle beginning at 7.00 h. Food and water were freely available. All experiments were carried out in a quiet, diffusely lit room between 8.00 h and 12.00 h.

Each experimental group consisted of 8–16 naive animals per drug dose. The Ethics Committee of Medical Academy in Białystok, Poland, approved this work (No. 2003/17).

Drugs

Baclofen (Tocris Cookson, UK, Ltd.) was administered intraperitoneally (*ip*) at a dose of 0.25 mg/kg in a volume of 1 ml/kg [9, 11, 12], 30 min before the tests. L-2-amino-4-phosphonobutyrate (L-AP4) (Tocris Cookson, UK, Ltd.) was dissolved first in 5 µl of hydroxide solution (1 mM) and then filled up to a 100 µl volume with 0.9% sodium chloride (pH 7.4) and administered into the lateral ventricle of the brain (*icv*) through the implanted cannulas at a dose of 100 µg (546 nmol) [49] in the volume of 5 µl per rat, during 30 s, 20 min before the tests. Throughout the experiments, injections with each drug (or vehicle) or a combination of baclofen with L-AP4 were given once to each rat. The time of injection signifies the administration of baclofen, which was always given first.

Surgery

A week before the experiments, the polyethylene cannulas were implanted under 10% chloral hydrate (400 mg/kg, intraperitoneally, *ip*) anesthesia into the lateral brain ventricle (*icv*) at the following coordinates: depth: 4 mm from the surface of the skull, 2.5 mm to the right from the sagittal suture and 1 mm behind the coronary suture. Cannulas were fixed to the skull bones with acrylic Deltamed PM 16 glue (Chemical Factory, Oświęcim, Poland). The injections were made using a Hamilton microliter syringe with a 0.3 mm external diameter. After implantation, rats were housed individually. After termination of each experiment, all animals were sacrified, their brains were removed, and the sites of implantation of cannulas and injection were verified macroscopically after brain sectioning. Animals with inappropriate injection sites were not used for analysis.

Amnesia induced by hypoxia

Hypoxia was induced by placing rats in a glass chamber filled with a mixture of 2% O_2 and 98% N_2 [2] for 4 min, after which they were immediately transferred to air. The hypoxia was induced 30 min (baclofen group) or 20 min (L-AP4 or baclofen with L-AP4 groups) before placing animals in the open field and elevated plus-maze. In the passive avoidance test, hypoxia was induced once immediately after administration of drugs (see passive avoidance test).

Open field test

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 center of the floor and following 1 min of adaptation, 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 it and touched it with its nose.

Passive avoidance test

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, the 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 (0.5 mA, 3 s) delivered through the grill floor of the dark compartment (learning trial). The passive avoidance reaction was checked 24 h later. The rats were placed on the illuminated platform once more and the latency to enter the dark compartment was measured, but no electric foot shock was applied. The latency was recorded during 300 s. To determine the effect of drug treatment on acquisition according to the protocol proposed by Matthies [33], baclofen and L-AP4 were injected on the second day 30 min (*ip*) or 20 min (*icv*) before induction of avoidance. To evaluate consolidation, all drugs were given on the second day, immediately after completion of induction of passive avoidance and to determine their effect on retrieval, the tested drugs were administered on the third day, 30 min (baclofen) or 20 min (L-AP4), before the retention test. Between the behavioral sessions, all animals were kept in their home cages.

Plus-maze test

The maze (constructed of grey colored wooden planks) consisted of four arms (two open and two closed), each 50 cm long and 10 cm wide, positioned to form a square cross around a 10-cm central square; the closed arms had the walls, each 40 cm high. The open and closed arms were opposite to each other. The maze was elevated to a height of 50 cm from the floor. The maze was covered with a removable lid. The maze was illuminated by uniform lighting (60 W). Thus, animals were treated with either vehicle (control), baclofen or L-AP4 and 25 min or 15 min later (baclofen or L-AP4, respectively), each 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. [38]. Immediately after the pretest exposure, the 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 moving all four feet into one arm. An increase in the percentage of open arm entries and increase in percentage of time spent in the open arms indicates potential anxiolytic-like activity, as rats naturally prefer the closed arms.

Tab. 1. The effect of baclofen and L-AP4 given alone or in combination on acquisition, consolidation and retrieval in the passive avoidance test

Treatment	Re-entry latencies (s) [n]		
	Acquisition	Consolidation	Retrieval
Control/vehicle	33.08 ± 2.47 [12]	38.9 ± 4.4 [10]	26.75 ± 2.1 [12]
Baclofen	20.75 ± 4.2 [8]*	43.4 ± 4.58 [10]	28.63 ± 4.31 [11]
L-AP4	198.55 ± 25.21 [9]***	201.8 ± 23.0 [10]***	190.84 ± 23.11[13]***
Baclofen + L-AP4	53.86 ± 19.15 [7]	103.61 ± 3.9 [13]***ooo	22.69 ± 2.83 [13]
Hypoxia/vehicle	13.18 ± 1.51 [11]***	14.36 ± 2.65 [11]***	14.33 ± 1.67 [12]***
Baclofen/hypoxia	35.25 ± 6.49 [8]+	30.03 ± 10.3 [11] +	17.09 ± 3.4 [12]
L-AP4/hypoxia	21.2 ± 4.37 [10]	72.3 ± 4.57 [10] ++	12.0 ± 2.3 [11]
Baclofen + LAP4/hypoxia	20.1 ± 5.36 [10]	13.14 ± 1.95 [14] ###	137.4 ± 45.3 [10]++ oo#

Control and hypoxic rats were treated *ip* or *icv* with vehicle, *ip* with 0.25 mg/kg of baclofen, *icv* with 100 µg of L-AP4 or with combination of baclofen with L-AP4. Values are the means ± SEM. * $p < 0.05$, *** $p < 0.001$ compared to control + vehicle group; oo $p < 0.01$, ooo $p < 0.001$ compared to baclofen group without hypoxia; + $p < 0.05$, ++ $p < 0.01$ compared to hypoxia + vehicle group; # $p < 0.05$, ### $p < 0.001$ compared to baclofen + L-AP4 group (Mann-Whitney test)

Body temperature measurement

Temperature was measured by means of a digital thermometer (Omron Flex, MC-205-E, Netherlands) with the temperature measurement range between 32–43.9°C. The thermometer was inserted into the rectum of the rat to a constant depth of 1 cm until a stable temperature reading was obtained [23]. The baseline body temperature was the mean of consecutive temperature measurements in each animal, i.e. immediately before the injection (vehicle, baclofen, L-AP4, or baclofen in combination with L-AP4). The temperature was measured at the following time points: 4 min after the drug injection, i.e. immediately after hypoxia induction, and 15, 20, 25, and 30 min after the injection.

Data analysis

The statistical significance of the results was computed by two-way analysis of variance (ANOVA) followed by the Newman-Keuls test, except for passive avoidance behavior, which was assessed with the Mann-Whitney rank 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.

Results

The effect of baclofen, L-AP4 and baclofen given with L-AP4 on acquisition in the passive avoidance test in rats (Tab. 1)

Re-entry latency was significantly shortened by baclofen but prolonged by L-AP4. Joint administration of baclofen with L-AP4 did not influence acquisition. Hypoxia significantly decreased the latency of the passive avoidance response and this effect was conspicuously reversed by baclofen only.

The effect of baclofen, L-AP4 and baclofen given with L-AP4 on consolidation in the passive avoidance test in rats (Tab. 1)

In the group of rats without hypoxia, no difference was found in latency to enter the dark compartment after administration of baclofen, but L-AP4 given alone or with baclofen significantly increased the avoidance response and changed the effects of baclofen.

The latency to enter the dark compartment was significantly shortened in rats after hypoxia but this effect was reversed by baclofen or L-AP4. Hypoxia de-

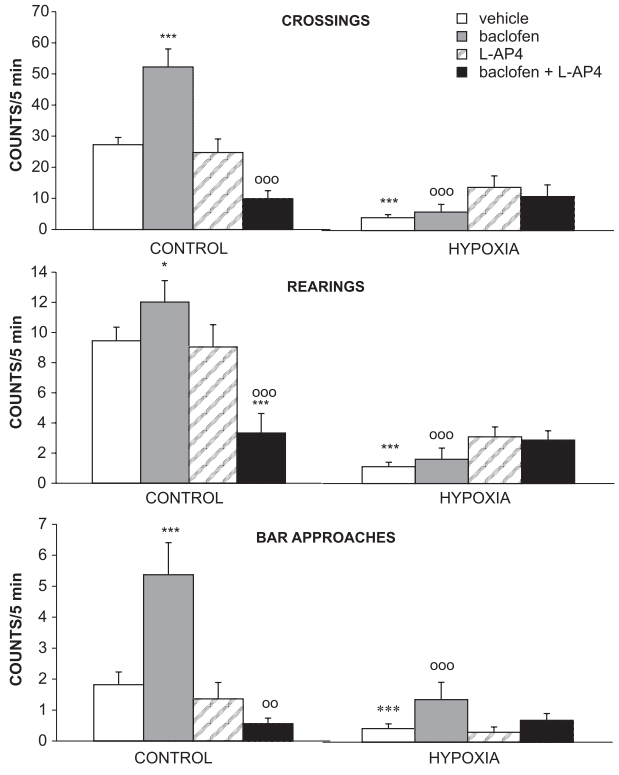

Fig. 1. The effect of baclofen and L-AP4 given alone or in combination on the number of crossings, rearings and bar approaches in the open field test. Columns represent the means ± SEM of the values obtained from 9–14 subjects. Baclofen (0.25 mg/kg, *ip*) was injected 30 min, L-AP4 (100 µg *icv*) 20 min before the test * $p < 0.05$, *** $p < 0.001$ compared to control + vehicle group, ^{oo} $p < 0.01$, ^{ooo} $p < 0.001$ compared to baclofen group without hypoxia; Crossings $F(7,75) = 17.981$, Rearings $F(7,75) = 11.760$, Bar approaches $F(7,75) = 5.974$ (ANOVA and Newman-Keuls tests)

creased the effect of the joint administration of baclofen with L-AP4 *vs.* the respecive group without hypoxia.

The effect of baclofen, L-AP4 and baclofen given with L-AP4 on retrieval in the passive avoidance test in rats (Tab. 1)

Neither baclofen given alone nor with L-AP4 influenced the avoidance response in rats without hypoxia. L-AP4 significantly prolonged the time spent on the illuminated platform.

Hypoxia markedly shortened the latency in rats and this effect was significantly attenuated by co-administration of baclofen with L-AP4. L-AP4 changed the effect of baclofen. Hypoxia enhanced the effect of joint administration of baclofen with L-AP4 *vs.* the respective group without hypoxia.

The effect of baclofen, L-AP4 and baclofen given with L-AP4 in the open-field test in rats (Fig. 1)

In rats without hypoxia, baclofen significantly enhanced the number of crossed fields, rearings and bar approaches. L-AP4 did not influence the behavior of the animals in the open field test. Baclofen given with L-AP4 inhibited rearings *vs.* the vehicle-treated group

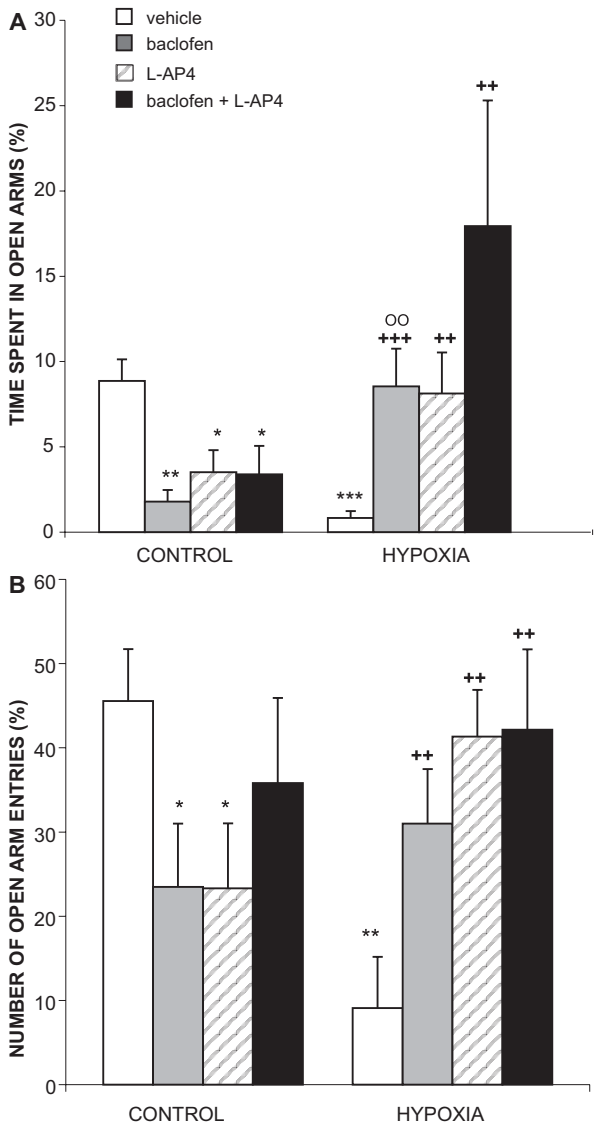

Fig. 2. The effect of baclofen and L-AP4 given alone or in combination in the elevated plus-maze test. Columns represent the means ± SEM of the values obtained from 10–12 subjects. Baclofen (0.25 mg/kg, *ip*) was injected 30 min, L-AP4 (100 µg *icv*) 20 min before the test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control + vehicle group; ^{oo} $p < 0.01$ compared to baclofen group without hypoxia; $^{++}$ $p < 0.01$, $^{+++}$ $p < 0.001$ compared to hypoxia + vehicle group; Time spent in open arms (%) $F(7,82) = 3.748$, Number of entries to open arms (%) $F(7,82) = 2.716$ (ANOVA and Newman-Keuls tests)

and crossings, rearings and bar approaches *vs.* the baclofen-administered groups of rats. L-AP4 diminished the effects of baclofen. Hypoxia produced a significant decrease in crossings, rearings and bar approaches. Neither baclofen nor L-AP4, given alone or with baclofen, influenced hypoxia-induced hypolocomotion. Hypoxia reduced the acitvity of baclofen *vs.* the respective group of rats without hypoxia.

The effect of baclofen, L-AP4 and baclofen given with L-AP4 in the elevated plus-maze test (Fig. 2)

In the control vehicle-treated rats of the total time (287 s) spent in either type of arms, 8.9% was spent in the open arms and 45.5% of entries were made into the open arms. The treatment of rats with baclofen or L-AP4 significantly reduced the percentage of the time spent in open arms and the percentage of entries into open arms. Baclofen administered with L-AP4 significantly decreased the percentage of time spent in open arms *vs.* the control vehicle-administered group of rats.

In hypoxic vehicle-treated rats of the total time (288 s) spent in either type of arms, 0.9 % was spent in the open arms and 9.1% of entries were made into the open arms (Fig. 2). These parameters significantly differed *vs.* the control vehicle-treated rats. Baclofen given to rats that had undergone hypoxia significantly increased the time spent in the open arms by up to 8.5%, and entries into the open arms by up to 31% *vs.* the hypoxic vehicle-administered group. In rats after hypoxia, L-AP4 significantly increased the time spent in the open arms by up to 8.1% and entries into the open arms by up to 41.3% *vs.* the hypoxic vehicle-given group. Baclofen applied with L-AP4 significantly increased the percentage of time spent in open arms as well as the percentage of entries into open arms in comparison with the group of hypoxic vehicle-treated rats. L-AP4 did not change the effects of baclofen as estimated in the elevated plus-maze test in rats.

The effect of baclofen, L-AP4 and baclofen given with L-AP4 on body temperature

The baclofen-treated group of rats had a lower overall body temperature within 4 min (36.52 ± 0.37°C) than the control vehicle-treated (37.83 ± 0.19°C) group of rats ($p < 0.01$). L-AP4 given alone or with baclofen did not influence body temperature. Immediately after induction of the amnesia (4 min) the hypoxic vehicle-administered rats had a significantly lower body temperature (37.0 ± 0.26°C) than the control vehicle-given (37.83 ± 0.19°C) animals at the same time ($p < 0.01$) (data not shown).

Discussion

In the present and earlier studies [2, 5, 6, 9, 15], hypoxia induced impairment of acquisition, consolidation and retrieval processes in the passive avoidance test. Learning and memory deficits induced by hypoxia are the result of dysregulation of many neurotransmitter systems, but the most significant are disturbances in the balance between the glutamatergic and GABAergic systems. The massive release of glutamate is excititoxic, particularly in the hippocampus, which is very sensitive to hypoxia and important for the learning and memory processes, and in synaptic plasticity [46, 47]. It has been suggested that drugs which reduce the activity of the glutamatergic system possess neuroprotective activity. We earlier reported that AIDA, an antagonist of group I mGluRs [7], 2R,4R-APDC [11], and L-AP4 [10], agonists of group II and III mGluRs, respectively, had a beneficial effect on the learning and memory processes in passive avoidance impaired by hypoxia. The presynaptic activity of 2R,4R-APDC results in reduction in glutamatergic transmission and this agonist of group II mGluRs may interact with baclofen on acquisition of passive avoidance in rats that had undergone hypoxia (unpublished data). In the present study, we tested the hypothesis that reduction of glutamate release by influencing the presynaptic $GABA_B$ receptors and group III mGluRs is important for learning and memory, especially in amnesia.

Baclofen, an agonist of $GABA_B$ receptors, impaired acquisition in the passive avoidance situation, had no effect on consolidation and retrieval (see also previous studies) [8, 9, 12, 13] but diminished hypoxia-induced acquisition and consolidation deficits. The effects of baclofen in the passive avoidance test are confusing. Zarrindast et al. [50] showed that low doses of baclofen (0.125 mg/kg and 0.25 mg/kg) showed only a tendency (without significance) to impair acquisition of passive avoidance in mice but diminished the improvement produced by physostigmine. Intracerebroventricular (0.25–2 μg) [51, 53] or intrahippocam-

pal (0.25–2 μg) [52] injection of baclofen dose-dependently reduced memory retention. However, Sharma and Kulkarni [43] found that baclofen enhanced memory acquisition in the passive avoidance paradigm in mice treated with scopolamine. In the hippocampus, baclofen can control the balance between glutamate and GABA [4], promote the induction of LTP, and participate in memory formation, both in acquisition and consolidation. Probably, baclofen may diminish the impairment of $GABA_B$ receptors observed immediately following ischemia [19] and in this way it readily blocks the presynaptic release of glutamate, and protects neurons from excitotoxicity. The beneficial effect of baclofen on learning appears to be revealed in anmesia.

L-AP4, an agonist of group III mGluR at a dose of 100 μg *icv* (546 nmol) enhanced the acquisition, consolidation and retrieval processes in the group of rats without hypoxia but improved consolidation of passive avoidance only in rats after hypoxia. Zalewska et al. [49] described that L-AP4 used at a dose of 100 μg *icv* in male rats without hypoxia produced similar effects as we obtained in the present study, but L-AP4 at a dose 50 μg *icv* (273 nmol) improved only consolidation and its dose of 75 μg *icv* (409.5 nmol) enhanced acquisition and consolidation in the passive avoidance behavior. It seems that 100 μg *icv* is the minimal dose producing beneficial effects in passive avoidance paradigm. Fedosiewicz et al. [18] found that L-AP4 at a dose of 400 nmol (5 μl of 80 mM solution) did not influence the learning and memory of the passive avoidance test. In our previous study, we showed that L-AP4 administered *icv* at doses of 10 and 25 μmol per rat impaired retention of passive avoidance [12]. Holscher [25] suggested that L-AP4 (5 μl of 10 mM solution) injected after training produced an amnestic effect in chicks tested in a one-trial passive avoidance task. Local intrahippocampal infusion of L-AP4 induced memory facilitation of step down inhibitory avoidance [48]. It seems that the behavioral effect of L-AP4 is strongly dependent on the applied dose, the way of administration and the kind of passive avoidance test. We suggest that a possible mechanism of the beneficial effect of L-AP4 may consist in serving as a filter that increases the signal-to-noise ratio for synaptic transmission [26], may regulate "metaplasticity phenomenon" [30] and at the dose given in the present study, it may enhance the activity of the dopaminergic system [49] and thus may contribute to improving the learning and memory processes [34]. L-AP4 at the applied dose probably activates mGluR4 localized presynaptically [39]. The significantly increased level of mGluR4 expression in the hippocampal region [27] or increased receptor affinity for the agonist during hypoxia may lead to a fall in the glutamate level that indicates the neuroprotective effects of L-AP4 mediated through this mechanism [3]. Because hippocampal formation is essentially damaged by hypoxia and is known to be involved in consolidation of memory [46, 47], it would seem that L-AP4 may have neuroprotective activity in this structure. Zhou et al. [54] found that rats which underwent diffuse brain injury, when treated with L-AP4 (10 μl of 100 mM *icv*) had a smaller number of damaged neurons as well as better motor and cognitive performance in behavioral tests.

Baclofen administered with L-AP4 enhanced consolidation and L-AP4 changed the effects of baclofen on this process but in rats that had undergone hypoxia, we observed an improvement of retrieval in the passive avoidance test. L-AP4 was shown to produce stronger inhibition of GABAergic transmission [21, 31, 40, 41] (85–90% of all GABAergic neurons), than of glutamatergic transmission in many brain areas, including those thought to be involved in learning and memory, such as the amygdala and the hippocampus, in which both types of receptors, $GABA_B$ and group III mGlu are present. The effect of both these drugs on consolidation suggests that the activity of L-AP4 is dominant in this process. This confirms the hypothesis that cooperation between the glutamatergic and GABAergic systems by acting at the presynaptic receptors [31] may be of importance during the learning and memory processes. Baclofen and L-AP4 may interact through presynaptic receptors on the dopaminergic neurons because L-AP4 diminishes baclofen effect on the locomotor activity. It seems that the dopaminergic system is of significance to our results but the problem how it was precisely affected requires additional study. The beneficial effects of baclofen and L-AP4 on retrieval in rats after hypoxia may be mediated *via* the ability of the used agonists to reduce glutamate and GABA release [16, 20], and synchronicity of the neuronal network. The obtained results may suggest that memory deficit is especially sensetive to the release of glutamate and GABA mediated by group III mGluRs.

Hypothermia induced by baclofen and by hypoxia, as obtained in the present work, probably inhibits dopamine, GABA and glutamate release [37, 45]. On the other hand, a low dose of baclofen potentiates behav-

ior related to nigrostriatal dopamine neurons [14], and we observed baclofen-induced hyperlocomotion.

Changes in motor activity and anxiety may influence aversively motivated behavior like passive avoidance. Motor function was measured in the open field test. In the present study, hyperlocomotion induced by baclofen may influence only the acquistion process. The motility produced by baclofen and L-AP4 probably did not participate in the observed effect on the memory processes without or after hypoxia. The anxiogenic-like activity, manifested by the lower percentage of time the rats spent in the open arms and the lower percentage of entries into the open arms in the elevated plus-maze test produced by baclofen given alone or with L-AP4 did not interfere with the effects observed in the passive avoidance test. The anxiogenic-like effect of L-AP4 may have affected the improvement of retrieval noted in the group of rats L-AP4-treated. Neither baclofen or L-AP4 given alone nor when given together, influenced hypoxia-induced inhibition of motility but they diminished the hypoxia-induced anxiogenic-like effect. The beneficial effect of baclofen given with L-AP4 on retrieval seems independent of non-specific influences, such as motor and anxiety. More experiments are required to clarify the exact mechanism(s) involved in the interaction between baclofen and L-AP4.

In conclusion, L-AP4 may be involved in cognition by influencing the action of $GABA_B$ receptor on consolidation in normoxic rats and on retrieval in rats after hypoxia. These effects support the hypothesis that a balance between the activities of group III mGluRs and $GABA_B$ receptors, probably by modulating the release of neurotransmitters, influences learning and memory and hypoxia-induced behavioral deficits.

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