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Olfactory bulbectomy and amitriptyline treatment influences mGlu receptors expression in the mouse brain hippocampus

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Abstract:
Olfactory bulbectomy (OB) is an established animal model of depression that has been investigated mostly in rats. As in human major depression, OB induces behavioral alterations that can be ameliorated by chronic antidepressant treatment. Furthermore, it was shown that OB induces changes of various protein receptor levels in brain areas that are important in antidepressant therapy. In the present study, we investigated the effects of OB and amitriptyline (AMI) treatment on the expression of metabotropic glutamate receptors (mGluR) in the mouse hippocampus using the western blot method. AMI was given for 14 days, in a dose of 10 mg/kg, intraperitoneally. The levels of most subtypes of mGlu receptors, e.g., mGlu1a (mGluR group I), mGlu2/3 (mGluR group II), mGlu4, and mGlu7 (mGluR group III) receptors, were measured. Additionally, immunohistochemical stainings were made in slices of the mouse hippocampus.
It was found that OB induced an increase in mGluR1a-immunoreactivity (IR), which was abolished by AMI treatment in the hippocampus. The removal of the olfactory bulbs caused a decrease in the level of mGlu2/3 receptors in the hippocampus, which was reversed after AMI administration. MGluR4-IR was decreased in the hippocampus in all the groups studied. A decrease of mGluR7-IR was observed in the OB group, and the effect was abolished by the administration of AMI. However, decreases in the level of mGlu2/3 and mGlu7 receptors were observed after AMI administration.
The results obtained indicate an influence of OB on mGlu receptors levels in the hippocampus, and the OB-induced effect can be reversed by chronic AMI treatment in the case of mGlu1a, mGlu2/3 and mGlu7 receptors.

Key words:
olfactory bulbectomy, depression, amitriptyline, mGluR, immunoblotting

Abbreviations: AMI – amitriptyline, CA – commisure anterior, DG – dentate gyrus, IMI – imipramine, KO – knock-out, mGluR – metabotropic glutamate receptors, OB – olfactory bulbectomy

Introduction

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system (CNS), and it

acts *via* ionotropic (iGluR-i.e NMDA, AMPA, kainate) and metabotropic glutamate receptors (mGluR). mGlu receptors comprise a family of at least eight receptors (with their splice variants) coupled to G-proteins linked to a variety of second-messenger pathways (for review see [6, 36]). They are classified into three groups according to their sequence homology, pharmacology and the transduction pathway they activate. Group I mGlu receptors include mGlu1 and mGlu5 receptors, group II comprises mGlu2 and mGlu3 receptors, and group III consists of mGlu4, mGlu6, mGlu7 and mGlu8 receptors [28, 29].

The role of the glutamatergic system in depression has been extensively investigated. Antidepressant-like activity was found after the administration of functional antagonists of NMDA receptor (ACPC, dizocilpine, CGP 37849) both in olfactory bulbectomized (OB) animals and in other models detecting antidepressant-like activity [10, 24, 30]. Recently, a combined administration of imipramine (IMI), a commonly used antidepressant drug, with NMDA receptor antagonist amantadine, was shown to be effective in treatment-resistant unipolar depression [33]. However, the high activity blockers of NMDA receptors often cause profound adverse reactions, such as psychotomimetic effects, memory dysfunction, ataxia, neurodegeneration and drug dependence [25, 35], which make the ligands of this receptor an unlikely therapeutic goal.

In recent years, we have focused our attention on ligands for mGlu receptors. mGlu receptors exert a modulatory role in the CNS. Therefore, it is possible that substances acting on mGlu receptors might be safer neuropsychiatric drugs, free of adverse effects and with a reasonably fast onset of action. The antidepressant-like effect of these ligands has been shown several times. Most studies indicate this effect for LY 367385 (an antagonist of mGlu1a receptors) [3], MGS0039 (an antagonist of mGlu group II receptors) [52], ACPT-1 (an agonist of group III receptors) [23], MPEP and MTEP (mGlu5 receptor negative modulators) [20, 49]; (for review see [22]).

Besides behavioral studies, there are reports on the influence of antidepressant therapy on the glutamate receptors in the brain [27]. Moreover, the regulation of the glutamatergic system by chronic IMI or electroconvulsive shock (ECS) was also observed in immunohistochemical studies of mGlu group I and mGlu group II receptors [1, 15, 40].

OB is one of the most validated animal models of depression [12]. It can be used not only for detecting antidepressant activity but also to explore the interrelationships between brain areas that are dysfunctional in patients suffering major depression [41]. Removal of the olfactory bulbs results in a series of neurochemical [8] and behavioral changes, such as increased hyperactivity and exploratory behavior [53] as well as significant impairments of learning- and memory-related behavior in a passive-avoidance test [42]. The latter are selectively reversed by chronic antidepressant treatment [12]. The neurochemical and biochemical mechanisms underlying the behavioral deficits in OB animals are far from clear [8]. There are some reports that alterations in the glutamatergic neurotransmission in OB animals were observed. It has been found that glutamate concentrations are decreased in the olfactory cortex of OB animals [5, 37]. The removal of olfactory bulbs also results in the decrease of NMDA receptor binding sites in discrete regions of the brain [9, 18, 31, 45].

Most of the studies with OB animals were performed on rats. Mice in the OB model have been investigated less often. Recently, it was shown that treatment with AMI as well as IMI, a typical antidepressants, was effective in reversing the behavioral deficits induced by OB ablation in mice at a level comparable to the effects seen in rats [11, 32].

Based on these results, we investigated whether prolonged administration of AMI induces any adaptive changes in expression and function of mGlu receptors in the mouse brain hippocampus. Therefore, two weeks after olfactory bulb removal, the OB and sham-operated mice were administered AMI for 14 days in a dose of 10 mg/kg, and then the levels of most subtypes of mGlu receptors in the hippocampus were measured.

Materials and Methods

Animals

Male C57BL/6J Han mice (2.5 months old, 20–25 g) were obtained from the Institute of Occupational Medicine (Łódź, Poland). Mice were group-housed in standard laboratory cages and kept in a temperature-controlled colony room (21 ± 2°C) with a 12-h light/dark

cycle (lights on at 7:00 a.m.). Commercial food and tap water were available *ad libitum*.

All experiments were carried out according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (revised 1996) and were approved by the Institute of Pharmacology, Polish Academy of Sciences in Kraków Animal Care and Use Bioethics Commission.

Animals were assigned to the following groups: a) sham-operated saline and b) sham-operated/antidepressant-treated mice as the control groups; c) OB C57/BL6 mice treated with saline and d) OB mice treated with an antidepressant drug.

Surgical procedure

Two weeks after arrival, a bilateral OB was performed according to the method described earlier [12, 50]. The animals were anesthetized with xylazine (6 mg/kg; *ip*; Biowet, Poland) in combination with ketamine (100 mg/kg; *ip*; Biowet, Poland) diluted in *aqua pro injectione* (Polpharma, Poland). An incision was made in the skin overlying the skull, and, after exposure of the skull, holes were drilled on both sides of the mid-line. Then the olfactory bulbs were removed by suction, and the holes were filled with hemostatic sponge in order to stop the bleeding. Sham-operated animals were treated in the same way except the bulbs were left untouched. The animals were given 14 days to recover following surgery prior to drug administration, and, during this period, they had daily handling with the experimenter to eliminate any aggressiveness that could otherwise arise. The subsequent two-week period allowed the consolidation of antidepressant drug efficiency [12, 43, 44, 50].

Drug treatment

Two weeks after the surgery, drug treatment began. AMI (amitriptyline, Sigma, USA) was given as an antidepressant drug in a dose of 10 mg/kg, *ip* for 14 days. Control animals received injections of distilled water in a volume of 10 ml/kg.

Histological analysis and tissue dissection

After two weeks of drug treatment and 24 h after the last dose of AMI animals were decapitated. Brains were removed and the lesion was estimated macroscopically immediately after brain removal; all brains with incomplete lesion or cortex damage were discarded from the experiment.

The hippocampi were dissected from the brains, and then these tissues were processed for Western blotting analysis.

Immunohistochemistry

Confocal microscopy analysis were performed to visualize localization of mGlu receptors in the hippocampal slices of control, non-operated and non-treated mice. The animals under deep vetbutal anesthesia were perfused through the ascending aorta with physiological saline, followed by cold phosphate-buffered 4% paraformaldehyde, as described previously [40, 46]. The brains were removed and postfixed for 24 h at 4°C. The brains were then cut into 50 μm frontal sections on a vibratome, at the levels containing the hippocampus. Free-floating sections were incubated for 48 h at 4°C in anti-mGlu receptors antisera (mGlu1a, mGlu2/3, mGlu4 and mGlu7 receptors). All antibodies were used in a dilution of 1:600. After incubation with anti-mGlu receptors antibody, the sections were rinsed in phosphate-buffered saline (PBS) and then incubated with secondary TexasRed anti-rabbit antibodies conjugated with Cy3 diluted 1:500. The specificity of immunostaining was controlled by omitting the primary antiserum. Stained slices were placed on Super Frost Plus microscopy slides (Menzel-Glaser, Germany), washed, and covered with fluorescent mounting medium (Vector Laboratories). Analysis were performed with the use of a DM RXA2 TCS SL confocal laser scanning microscope, using a 63×/1.4–0.6 (HCX PL APO) oil objective and confocal software (Leica, Microsystems). The fluorescence was excited with a He/Ne laser emitting a line at 543 nm. The exposure settings and gain were identical for each image. The background noise of each confocal image was reduced by averaging four scans/line and six frames/image.

Western blotting procedure

After decapitation, the rats' brains were removed and cooled in ice-cold physiological saline; the hippocampi were carefully dissected. Tissue samples were frozen on dry ice immediately after dissection and stored at –70°C until the Western blotting analysis. The samples were homogenized at 4°C in 0.5 ml of homogenizing buffer containing 2% sodium dodecyl sulfate (SDS). The homogenates were boiled at 100°C

and cleared by centrifugation (1,000 *g* for 15 min at 4°C). Protein concentration in the supernatant was determined using the BCA Protein Assay kit (Sigma). Aliquots were adjusted to contain 50 mM Tris-HCl (pH = 6.7), 2% SDS, 4% glycerol, 2% di-thiothreitol with bromophenol blue as a marker (Novex Tris-Glycine sampling buffer, 2×). Samples were heated for 10 min at 95°C and then subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE, 7.5% acrylamide/bis-acrylamide) [13]. The amount of protein per lane was optimized in pilot studies, in which western blots were performed using different amounts of protein per sample extracted from the experimental brain region. For quantitative analysis of separate mGlu receptor proteins, we chose the amount of total protein for which twofold differences in protein content were linearly reflected by the assay. After the SDS-PAGE, the proteins were electrophoretically transferred to nitrocellulose membranes of 0.45 μm pore size (Sigma).

To control equal gel loading, gels were stained with Coomassie brilliant blue R250 after protein transfer, and the nitrocellulose membranes were stained with Ponceau S. The intensity of the staining was compared between lanes. The blots were blocked using 3% non-fat milk (Sigma) in Tris-buffered saline (TBS) and incubated with the primary antibody. After four 10 min washes in TBS with 0.1% Tween 20 (TBS-T), the blots were incubated with POD-conjugated secondary antibody anti-mouse (50 mU/ml) or anti-rabbit (20 mU/ml) for 1 h. After four 10 min washes in TBS-T, the signal was detected using the Lumi-Light^{PLUS} substrate (0.1 ml/cm^2) (Roche Applied Science) reagent and visualized by applying the Fujifilm LAS-1000 fluoroimager system. Relative levels of immunoreactivity (IR) were quantified using the Fujifilm software Image Gauge.

After mGlu receptors' signal visualization, the blots were washed several times and then incubated for 2 h with 1:20,000 dilution of mouse anti-βactin antibody (an internal standard) (Sigma) and then processed as described above.

The measurements were performed separately for each mouse.

Antibodies

The specific primary antibodies are presented in Table 1.

Statistical analysis

The data from the behavioral tests were analyzed using one-way ANOVA followed by a Newman-Keuls multiple comparision test. Statistical evaluations were done using the software STATISTICA 7.0.

Results

Immunohistochemical staining

All mGluR antibodies revealed specific patterns of IR in the mouse hippocampus. All of these immunostaining patterns were abolished by preadsorption of the antibodies with the homologous antigens (data not shown). The specificity of the immunostaining for mGlu receptors was also verified by omitting the primary/secondary antibodies.

For the group I mGlu receptors, we used the antibodies reactive to the mGlu1a receptor splice variant. In the hippocampi of control rats, the highest density of mGluR1a-IR nerve cell bodies and fibres was observed in the stratum oriens of the commisure anterior (CA1–CA2) hippocampal region and in the hilus of the dentate gyrus (DG). Additionally, IR was seen in interneurons scattered above and below the pyramidal layer in the stratum oriens, lucidum and radiatum (es-

Tab. 1. Summary of the concentrations, combinations and sources of primary and secondary antibodies (Ab)

Ab against	Species (raised in)	Dilution	Blocking	1st Ab incubation	2nd Ab incubation	Source of
mGluR1a	Rabbit (polyclonal)	1 μg/ml	2 h RT	1 h RT	1 h RT	Chemicon
mGluR2/3	Rabbit (polyclonal)	2 μg/ml	2 h RT	1 h RT	1 h RT	Upstate Cell Signalling
mGluR4	Rabbit (polyclonal)	2 μg/ml	24 h 4°C	2 h RT	1 h RT	Chemicon
mGluR7	Rabbit (polyclonal)	1 μg/ml	1 h RT	18 h 4°C	1 h RT	Upstate Cell Signalling
βactin	Mouse (monoclonal)	1 μl/20 ml	6 h RT	2 h RT	1 h RT	Sigma

pecially in the CA3 region); some IR neurons were also found in the stratum lacunosum-moleculare. Few stained cells could only be seen in the pyramidal cell layer. In the CA3 region, a delicate network of mGluR1a-positive fibres was observed in the stratum lucidum.

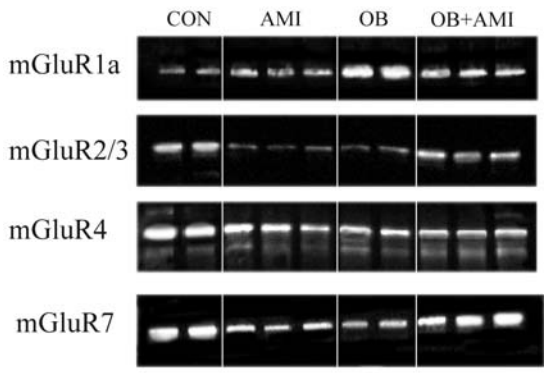

Fig. 1. Representative blots of mGlu1a, mGlu2/3, mGlu4 and mGlu7 receptors in the hippocampus of control (CON), amitriptyline (AMI), olfactory bulbectomy (OB) and OB+AMI treated groups

For the group II mGlu2/3 receptors, we used the antibody reactive to both mGlu2 and mGlu3 receptors. The density of the mGlu2/3 receptors was highest in the neuropil of the CA1 field of the hippocampus. In the CA3 area, labeling was stronger in the inner than in the outer layer of the stratum lacunosum moleculare, and, in the DG, it was stronger in the middle than in the outer one-third of the molecular layer.

Among the group III receptors, mGlu4 receptors were seen in the hippocampus as strongly stained neurons, mainly in the CA1 and DG field. mGlu7 receptor labeling was seen as neuropil in the CA1 area of the hippocampus and in the DG, where associational/commissural fibers were seen. mGlu7 receptors were also seen as weakly stained nerve terminals and processes (Fig. 7).

Western blot analysis

Immunoblots showed one monomeric band with mGlu1a receptor (140 kDa), mGlu2/3 receptor (108 kDa) and

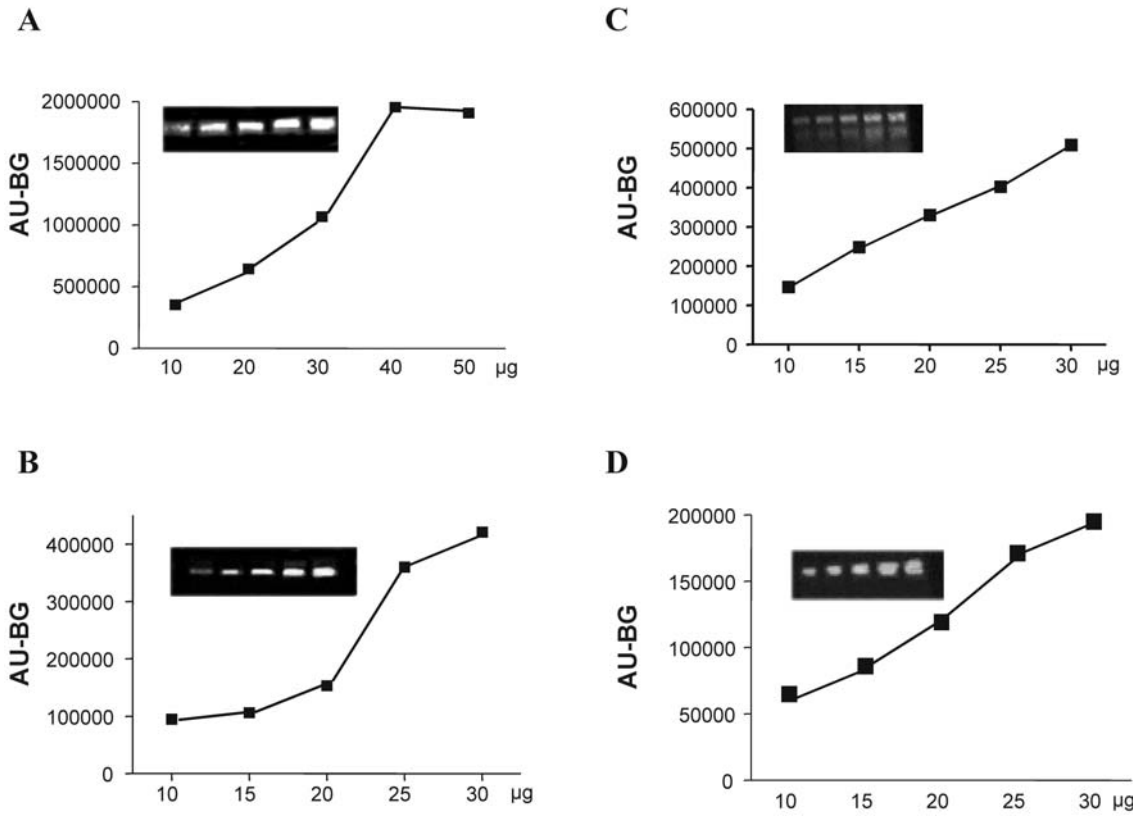

Fig. 2. Graph and photomicrographs showing that the optical density values for anti-mGlu1a **(A)**, anti-mGlu2/3 **(B)**, anti-mGlu4 **(C)** and anti-mGlu7 **(D)** receptors are linear with protein concentration

mGlu7 receptor (97 kDa) corresponding to the receptor monomer. Two different bands were recognized by anti-mGlu4 receptor (about 100 kDa). Examples of immunoblots of protein extracts from the hippocampus of control, OB and antidepressant-treated animals are shown in Figure 1. The amount of protein per lane was optimized in pilot studies, in which western blots were performed using different amounts of protein per sample extracted from the experimental brain region. The amount of protein taken is shown on the graphs in Figure 2.

OB caused a significant increase in the level of mGlu1a receptor to about 162% of the control value (Fig. 3). A decrease of mGlu2/3 receptor protein by 40% (Fig. 4), of mGlu4 receptor by 21% (Fig. 5), and of mGlu7 receptor by 63% (Fig. 6) of the control levels was observed. Chronic AMI treatment (10 mg/kg) did not influence the level of mGlu1a receptor, but it

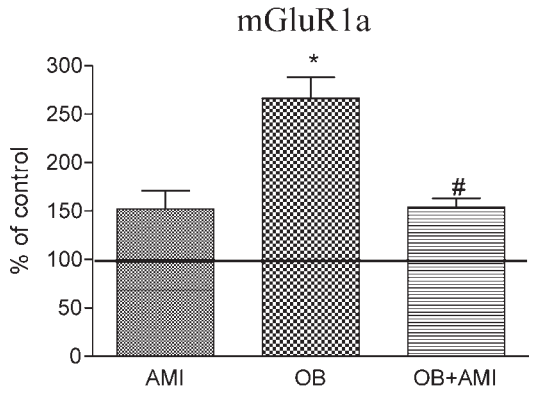

Fig. 3. The effect of olfactory bulbectomy (OB) and chronic amitriptyline (AMI) treatment on mGlu1a receptor protein expression in the hippocampus of the mouse brain. Results (mean ± SEM) are shown as a percentage of control mGluR:βactin ratio. N = 8 for each group. One-way ANOVA followed by Newman-Keuls *post-hoc* comparision revealed statistical significance of OB effect and significant OB+AMI interaction; where $F(2, 28) = 5.796$, * $p < 0.01$ relative to control and $^{\#}$ $p < 0.05$ relative to OB

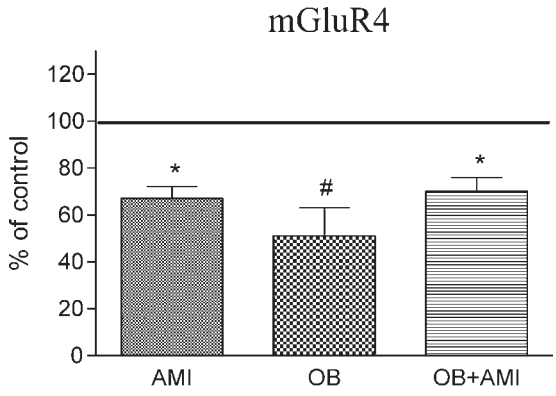

Fig. 5. The effect of olfactory bulbectomy (OB) and chronic amitriptyline (AMI) treatment on mGlu4 receptor protein expression in the hippocampus of the mouse brain. Results (mean ± SEM) are shown as a percentage of control mGluR:βactin ratio. N = 8 for each group. One-way ANOVA followed by Newman-Keuls *post-hoc* comparision revealed statistical significance of AMI, OB and OB+AMI effect and significant OB + AMI interaction; $F(2, 28) = 5.211$, $^{\#}$ $p < 0.01$ and * $p < 0.001$ relative to control

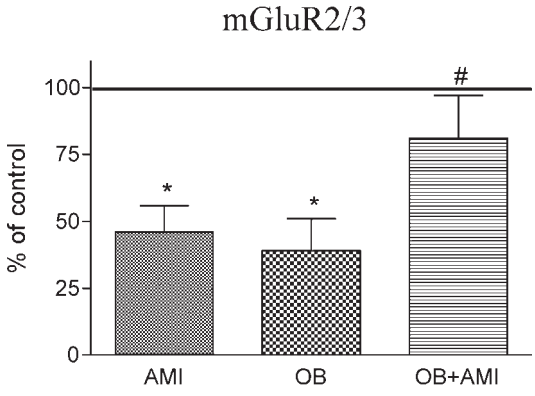

Fig. 4. The effect of olfactory bulbectomy (OB) and chronic amitriptyline (AMI) treatment on mGlu2/3 receptor protein expression in the hippocampus of the mouse brain. Results (mean ± SEM) are shown as a percentage of control mGluR:βactin ratio. N = 8 for each group. One-way ANOVA followed by Newman-Keuls comparision revealed statistical significance of AMI, OB and OB+AMI effect and significant OB + AMI interaction; $F(2, 28) = 16.58$, * at least $p < 0.001$ relative to control and $^{\#}$ $p < 0.01$ relative to OB group

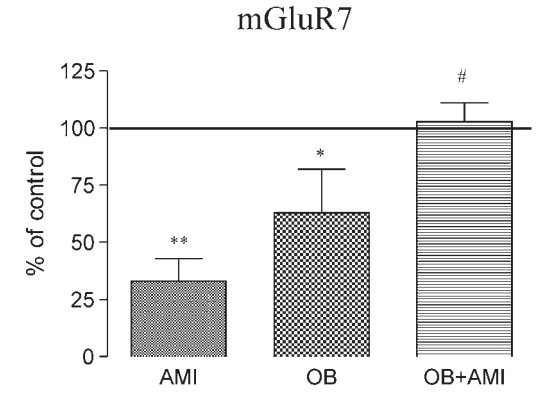

Fig. 6. The effect of olfactory bulbectomy (OB) and chronic amitriptyline (AMI) treatment on mGlu7 receptor protein expression in the hippocampus of the mouse brain. Results (mean ± SEM) are shown as a percentage of control mGluR:βactin ratio. N = 8 for each group. One-way ANOVA followed by Newman-Keuls comparision showed a significant AMI, OB and OB + AMI effect and significant OB+AMI interaction; $F(2, 28) = 16.114$, ** $p < 0.01$ relative to control, * $p < 0.05$ relative to control and $^{\#}$ $p < 0.05$ relative to AMI-treated group

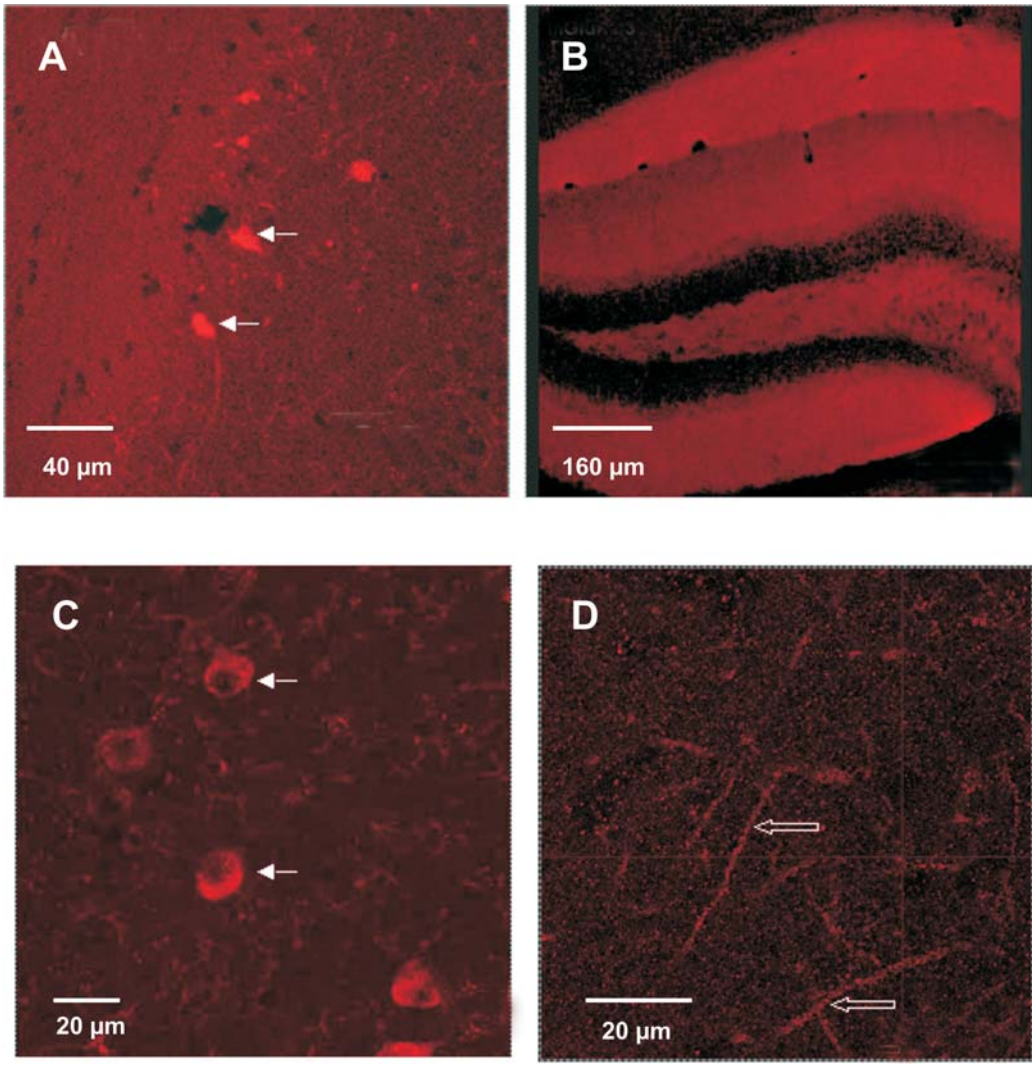

Fig. 7. The examples of immunoreactivity for five mGlu receptors in mouse hippocampus. Parasagittal sections through the hippocampus were reacted with antibody to mGlu1a **(A)**, mGlu2/3 **(B)**, mGlu4a **(C)**, mGlu7 **(D)** receptors. White arrows indicate nerve cells bodies and empty arrows indicate nerve terminals

did cause a significant decrease in the mGlu2/3 receptor level to 50%, in the mGlu4 receptor protein level to 61%, and in mGlu7 receptor level to 36% as compared to sham-operated animals. When AMI was administered to the OB mice, it normalized the OB-induced increase in mGluR1a-IR (Fig. 3). The levels of both mGlu2/3 and mGlu7 receptors were also normalized (Fig. 4 and Fig. 6). Only the mGlu4 receptor level remained unchanged by AMI administration in OB animals (Fig. 5).

No changes in the βactin level, which was used as an internal standard protein, were detected in experiments performed in the hippocampus after OB and prolonged AMI treatment.

Discussion

In our previous studies, we showed that the antidepressant drugs IMI and citalopram or electroconvulsive shock regulated the mGlu1a, mGlu5a, mGlu4 and mGlu7 receptor protein levels in the hippocampus and/or in the cortex of the rat brain [40, 48]. We also showed that mGlu5 receptors are regulated (increased in CA1 and decreased in the CA3 region of the hippocampus) when measured in one of the most validated animal models of depression, e.g., chronic mild stress [46].

Therefore, we decided to investigate the effect of antidepressant treatment on the IR of selected mGlu receptors of all three groups of mGlu receptors. We chose the hippocampus since this brain region is generally accepted to play an important role in mood disorders [32, 34]. An elevated level of corticosterone, a chronic stressor, caused an increase of glutamate in that structure, further indicating an important role of the hippocampus in regulating stress-related disorders [39]. Moreover, it had already been shown, both in our laboratory and by other scientists, that the mGlu receptors are particularly abundant in that structure [38].

OB is well known and one of the most validated animal models of depression. The first study concerning the role of antidepressant therapy in OB rats was published in 1976 [44]. Since then, a number of behavioral, biochemical and pharmacological investigations have been done. However, this is the first complex study measuring specific changes in the expression of individual mGlu receptor subtypes, both in the OB mice and after treatment of OB animals with AMI, a classic tricyclic antidepressant drug that, in our hands, reversed several effects of bulbectomy.

Our immunohistochemical observations about the distribution of mGlu receptors in the hippocampus are generally in agreement with those obtained earlier by Shigemoto et al. [38]. The IR of all mGlu receptors was localized mainly in the neuropil as well as on the nerve terminals and dendrites of the hippocampal neurons. Immunostaining was observed in the nerve cell bodies mainly in the case of mGlu4 receptors. A few nerve cell bodies were seen also when mGlu1a receptors were stained. Therefore, the best method to quantify the changes of these receptor protein levels was western blotting rather than immunohistochemistry.

The most robust effect obtained in this study was the demonstration that the mGlu1a receptor protein level increased in the hippocampus after olfactory bulb ablation and that the effect was reversed after AMI administration. In the present study we did not performed mGlu5 receptor analysis. The only data concerning the role of the mGlu5 receptor in the action of antidepressant drugs were published in 2002 by Śmiałowska et al., where increases in the CA1 and CA3 field of the hippocampus were observed after 21 days of chronic IMI treatment; in fact, these results were obtained for one of the splice variants of mGlu5 receptor, mGluR5a [40]. An increase of mGlu5a receptors in the CA1 region and a decrease of the same receptors in the CA3 region of the hippocampus were observed in animals that underwent the chronic mild stress procedure [22, 47]. When we took into account the results of behavioral studies, the antagonists of mGlu1 (EMQMCM) and negative allosteric modulators of mGlu5 receptors (MPEP, MTEP) exerted antidepressant-like activity in the forced swim test (FST), tail suspension test (TST) and in the OB model of depression [2, 14, 49]. All these data confirm the notion that receptors belonging to group I mGlu receptors may play an important role in the mechanism of the action of antidepressant drugs.

Concerning group II mGlu receptors, there are several reports about the role of their ligands, mainly antagonists, in behavioral tests. An antidepressant-like effect was observed for a selective group II antagonist MGS-0039 as well as the non-selective LY 341495 in the tail suspension test in mice [4, 51] and in the learned helplessness model [26, 52]. Neurochemical reports show that chronic (21 days) IMI treatment up-regulates the expression of mGlu2/3 receptor proteins in the hippocampus, nucleus accumbens, cerebral cortex and corpus striatum [15]. It was also shown that chronic IMI treatment reduced inhibitory properties of mGlu2/3 receptors [19]. The present results show the decrease of these receptors in the hippocampus after AMI administration. The variability may be due to species or drug differences in these two sets of studies as well as shorter time of administration (14 days). In the work of Matrisciano et al. [16], FSL rats (Flinders Sensitive Line, animals with a depressive-like phenotype) showed a selective reduction in the expression of mGlu2/3 receptors in the hippocampus, whereas expression was unchanged in the other brain structures. The OB in our experiments led to similar results. Moreover, the combination of AMI administration and OB normalized the level of mGlu2/3 receptors in our studies. The data support the notion of regulation of group II mGlu receptors by tricyclic antidepressant drugs.

The group III mGlu receptors comprise the largest family of mGlu receptor. In this study, we measured two subtypes of these receptors: mGlu4 and mGlu7. The mGlu6 receptor subtype was not measured as it is expressed mainly in the retina [17]. Our results show that the IR of mGlu4 receptors was diminished in the hippocampi from animals of all the experimental groups. This is in conflict with the results observed after IMI treatment in rats, where the level of mGlu4 receptors remained unchanged [15, 48]. The difference in species or drugs may account for the variability.

Tab. 2. Summary of the literature concerning the changes in the metabotropic glutamatergic receptor (mGlu) levels after antidepressant administration and/or in the chosen models of depression

	mGluR1	mGluR5	mGluR2/3	mGluR4	mGluR7
Electroconvulsive shock	↑ [40]	↑ [40]			
Imipramine	↔ [40]	↑ [40]	↓ [15]	↔ [48]	↔ [48]
Citalopram				↔ [48]	↓ [48]
Amitriptyline	↔ [present study]		↓ [present study]	↓ [present study]	↓ [present study]
Olfactory bulbectomy	↑ [present study]		↓ [present study]	↓ [present study]	↓ [present study]
Olfactory bulbectomy + amitryptiline	norm. [present study]			↓ [present study]	norm. [present study]
Chronic mild stress		↑ CA1 ↓ CA3 [47]			
Chronic mild stress + imipramine		↔ [Wierońska, unpublished]			
Flinder-sensitive line			↓ [16]		

↑ – increase; ↓ – decrease; ↔ – lack of changes; norm – normalization of the effects of olfactory bulbectomy after amitriptyline administration

The behavioral data from the group III mGlu receptor ligands showed that PHCCC, an allosteric modulator of mGlu4 receptor, enhances the antidepressant effect of ACPT-1, a non-selective agonist of group III receptors, but it does not exert such an effect when given alone, implying the possible involvement of mGlu receptors in depression [23].

Our results concerning the mGlu7 receptors show that they are diminished both after AMI administration and in OB animals. Similar to the results obtained with mGlu2/3 receptors, the level of mGlu7 receptors was normalized in OB animals after AMI administration, showing that certain subtypes of presynaptically placed receptors that may regulate the release of glutamate or GABA are influenced by AMI. It may be speculated that mGlu7 receptors play a role in the mechanism of the action of antidepressant drugs. Experiments with knock-out (KO) animals without the mGlu7 receptor gene were performed by the group of Cryan et al. [7]. They have revealed that mGluR7-deficient mice had antidepressant-like behavior in the FST and in the TST. In contrast, the antidepressant action of AMN082, a positive allosteric modulator of mGlu7 receptors, was demonstrated in wild-type mice, but it did not change the behavior of mGlu7 receptor KO mice compared to wild type littermates in the TST, while IMI, used as a reference control, significantly reduced their immobility, indicating an mGlu7 receptor-dependent mechanism of the antidepressant-like activity of AMN082 [21]. More detailed studies are needed to understand the role of mGlu7 receptors in the mechanism of the action of antidepressant drugs.

In conclusion, the present study points to the importance of mGlu receptors in depression as well as in the mechanism of the action of AMI, a commonly used tricyclic antidepressant drug. Our results show that the behavioral deficit observed in OB animals may be a result of the increase in mGlu1a and a decrease in mGlu2/3, mGlu4 and mGlu7 receptors. Prolonged administration of antidepressant restores expression levels in the case of mGlu1, mGlu2/3 and mGlu7 receptors, which may suggest a significant role of these receptors in the treatment of depression. In Table 2, we summarize the effects of antidepressant drugs on mGlu receptor reactivity in the hippocampus. Undubtedly, the results obtained in the case of the mGlu2/3 and mGlu7 are controversial and more studies are needed to explain the discrepancy. It is not clear how the effect of decreased IR of these receptors can be reversed by AMI administration when AMI itself caused diminish IR of these receptors. As it comes to mGlu2/3 receptors a possible explanation of such a result is that antibodies we had were reactive to both mGluR2 and mGlu3 receptors subtypes. The antidepressant-like effect of AMI and the bulbectomy-induced effect may be connected with changes in one type of these receptors. It is also possible that the AMI administration and bulbectomy regulate these two subtypes of mGlu receptors in the opposite direc-

tion. The detection of such changes is limited because of the lack of subtype-specific antibodies.

In the mice hippocampus mGlu7 receptors are present in two subtypes: mGlu7a and mGlu7b receptor, which slightly differ in the localization and sensitivity for ligands [38]. The response of particular subtype can be masked, as in the case of mGlu2/3 receptors, because the available antibody recognized both subtypes of receptors.

The results obtained in this study as well as the results obtained by other researchers lead us to speculate that mGlu receptors of all groups are involved in the pathology and pharmacotherapy of depression, as in several cases (mGlu1, mGlu2/3, mGlu7) treatment with AMI normalized the level of these receptors in OB animals.

It can be speculated that group I mGlu receptors that mediate the excitatory effect of Glu are up-regulated in the OB model of depression, whereas group II/III receptors that modulate inhibitory effects on glutamatergic transmission are down-regulated, which suggests that the state of depression might be connected with the hyperactivity of glutamatergic transmission.

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