Chronic imipramine treatment reduces inhibitory properties of group II mGlu receptors without affecting their density or affinity

Agnieszka Pałucha, Piotr Brański, Kinga Kłak, Magdalena Sowa

Department of Neurobiology, Institute of Pharmacology Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

Correspondence: Agnieszka Pałucha, e-mail: nfpaluch@cyf-kr.edu.pl

Abstract:
An increasing body of evidence indicates an important role of the glutamatergic system in the pathophysiology of depression. Not only ionotropic but also metabotropic glutamate receptors (mGlu receptors) have been suggested to be involved in the mechanism of action of antidepressant drugs. Moreover, several mGlu receptor ligands possess a great antidepressant potential. Group II mGlu receptor antagonists have been shown to induce antidepressant-like effects in rodents. An influence of chronic antidepressant treatment on group II mGlu receptors has also been suggested. In our studies, we examined an influence of repeated (21-day) imipramine treatment on the density of group II mGlu receptors and affinity of mGlu2 and mGlu3 receptor radioligand [3H]-LY341495 for group II mGlu receptors in the rat brain hippocampus and frontal cortex. Moreover, we analyzed an influence of chronic imipramine administration on the ability of group II mGlu receptor agonist, 2R,4R-APDC, to inhibit forskolin-stimulated cAMP accumulation in the rat brain cortical slices. We found that inhibitory properties of group II mGlu receptors were diminished after chronic, but not acute imipramine administration. However, no changes in the density or affinity of the mGlu2 and mGlu3 receptor ligand for group II mGlu receptors were observed.

Key words:
cAMP, imipramine, group II mGlu receptors

Abbreviations: 2R,4R-APDC – (2R,4R)-4-aminopyrrolidine-2,4-dicarboxylic acid, cAMP – 3’-5’-cyclic adenosine monophosphate, BDNF – brain-derived neurotrophic factor, CREB – cAMP response element binding protein, ECS – electroconvulsive shock, [3H]-LY341495 – 3H-2S-2-amino-2-(1S,2S-2-carboxycycloprop-1-yl)-3-(xanth-9-yl)propanoic acid, mGlu receptors – metabotropic glutamate receptors

Introduction

Antidepressant drugs acting by modulation of monoaminergic systems, such as imipramine, induce their therapeutic effects after long-lasting, few-weeks’ administration. Therefore, neuroadaptive changes induced by a prolonged treatment with antidepressants in different brain regions are considered to be related to the therapeutic, antidepressant effects, since they correlate with a clinical latency of antidepressants [10].

A number of pharmacological data indicated the involvement of the main excitatory system in the brain, glutamatergic system, in the pathophysiology of depression [13]. It has been found that functional NMDA receptor antagonists and AMPA receptor potentiators produce antidepressant-like effects in several tests and models of depression. An NMDA receptor an-
agonist, ketamine, elicited also an antidepressant effect in patients suffering from major depression, non-responding to classical antidepressants [1, 23]. Moreover, it was found that chronic treatment with antidepressant drugs or electroconvulsive shock (ECS), reduced NMDA receptor reactivity and function in rodents [17].

Our previous experiments have shown that mGlu receptors are also involved in the mechanism of action of antidepressant drugs. We have found that chronic application of antidepressants modulates the activity and/or expression of group I mGlu receptors (mGlu1 and mGlu5 receptors). There is some evidence that repeated electroconvulsive therapy (ECT) or prolonged imipramine treatment induced a subsensitivity of group I mGlu receptors in the hippocampus [14, 22]. It was also shown that chronic ECT and/or imipramine treatment produced significant changes in the expression of mGlu1a and mGlu5a receptors in the same region of the hippocampus [18]. Subsequent studies demonstrated antidepressant-like activity of group I mGlu receptor antagonists in the tests and models of depression (for review see [13]). An increase in hippocampal BDNF gene expression in rats after chronic treatment with a mGlu5 receptor antagonist, MPEP, confirmed antidepressant potential of this compound [7].

In the last few years, attention has focused on group II mGlu receptors (mGlu2 and mGlu3 receptors), which are highly expressed in brain structures apparently related to emotional states, including the forebrain and limbic areas, such as the amygdala, hippocampus and prefrontal cortex [19, 20]. Since group II mGlu receptors are negatively linked to the adenylyl cyclase signal transduction pathway [5] and are localized mainly at the extrasynaptic sites of terminal axons on glutamatergic neurons, they act as autoreceptors and are responsible for the decrease in the glutamate release, especially under conditions of glutamate excess in the synapse [16].

It has been shown that group II mGlu receptors may be involved in the mechanism of action of antidepressant drugs. Antagonists of group II mGlu receptors (MGS0039 and LY341495) have been shown to induce dose-dependent antidepressant-like effects in mice and rats [2, 21]. Moreover, Western blot analysis indicated that chronic imipramine treatment up-regulated the expression of mGlu2 and mGlu3 receptors in the hippocampus and several other regions related to depression [9]. Another study of the same research group investigated the influence of group II mGlu receptor ligands on the imipramine-induced adaptive changes in the hippocampus. The results showed that down-regulation of β-adrenergic receptors, which typically occurs after 21 days of imipramine treatment, appeared at shorter times when imipramine was combined with low doses (0.5 mg/kg, ip) of the selective mGlu2 and mGlu3 receptor agonist, LY379268, or antagonist, LY341495 (1 mg/kg, ip). Therefore, it seems that neuroadaptation to imipramine may be influenced by drugs that interact with group II mGlu receptors which are supposed to shorten the clinical latency of classical antidepressants [8].

Based on these observations, we decided to investigate further a potential involvement of group II mGlu receptors in the mechanism of action of antidepressant drugs by measuring the influence of chronic treatment with imipramine on the density of group II mGlu receptors (Bmax) or the affinity (Kp) of mGlu2 and mGlu3 receptor radioligand [3H]-LY341495 for group II mGlu receptors in the rat hippocampus and cerebral cortex.

Moreover, we examined the influence of repeated imipramine administration on the ability of the group II mGlu receptor agonist to inhibit forskolin-stimulated cAMP formation in rat cerebral cortical slices.

### Materials and Methods

#### Animals

Male Wistar rats weighing 180–200 g at the beginning of the experiment were used to investigate the influence of chronic treatment with antidepressant drugs on group II mGlu receptor function. The animals, obtained from the local breeding farm at the Institute of Pharmacology PAS, Kraków, Poland, were housed five per cage under standard laboratory conditions of lighting (light on from 06:00–18:00 h) and temperature (19–21°C). Food and water were freely available. Rats received imipramine freshly dissolved in sterile water twice daily (at 08:00 and 18:00) for 21 days (10 mg/kg, 2 ml/kg po) or acutely (10 mg/kg, 2 ml/kg, po). The controls received sterile water instead of imipramine. The rats were killed 48 h after the last dose of the drug. Eight rats per group were used in all experiments.
All procedures were conducted according to the guidelines of the National Institutes of Health Animal Care and Use Committee, and were approved by the Ethics Committee of the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Chemicals

The following agents were used: imipramine hydrochloride (Polfa, Poland), forskolin (Tocris Cookson Ltd., Bristol, UK), 2R,4R-APDC (Tocris Cookson Ltd., Bristol, UK), [3H]-adenine and [14C]-cAMP (NEN Life Science Products, Boston, USA), [3H]-LY341495 (Tocris Cookson Ltd., Bristol, UK).

Binding studies

Animals were killed 48 h after the last treatment, and their brains were removed. Dissected brain structures (frontal cerebral cortices and hippocampi) were frozen on dry ice and stored at −80°C. Hippocampi and frontal cortices were homogenized in 10 volumes of 30 mM Tris-HCl + 2.5 mM CaCl2 buffer (pH 7.6 at 5°C), then membranes were centrifuged at 20,000 × g for 10 min, the pellet was resuspended in the same volume of buffer and incubated for 30 min at 37°C followed by three more washes. The final pellet was resuspended in 10 volumes of buffer and frozen at −20°C. Pellets of rat brain homogenate were thawed on the day of assay and washed three times by centrifugation (20,000 × g, 10 min) with ice-cold assay buffer (10 mM KH2PO4 + 100 mM KBr, pH 7.6). The binding reaction was assayed by incubating membranes (0.03–0.06 mg of protein) in glass tubes in the presence of six concentrations of [3H]-LY341495 (0.2–7.0 nM) in assay buffer. Nonspecific binding was defined with 1 mM L-glutamate. Assay mixtures (final volume 500 μl) were incubated on ice for 30 min. The incubation was terminated by rapid filtration with ice-cold assay buffer using Brandel cell harvester through glass fiber Whatman GF/B filters prewet with the same buffer. The filters were washed three times and then transferred to scintillation vials with 4 ml of liquid scintillation cocktail. Vials were allowed to set for several hours prior to counting bound reactivity on a Beckman LS-6500 scintillation counter. Specific binding was defined as the difference between total and nonspecific binding, and is expressed in pmol/mg of protein. Protein concentration was determined using the Pierce Coomassie micro assay. Data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA).

Measurement of cAMP accumulation

Cross-chopped cerebral cortical slices (350 μm) were prepared from rat brains using McIlwain tissue chopper. The chopped tissue was suspended in oxygenated Krebs medium, pH 7.4. The formation of [3H]-cAMP in [3H]-adenine-prelabeled slices was assayed as described previously [12]. Briefly, after 15-min equilibration at 37°C in the gassed (O2/CO2, 95:5) Krebs medium, pH 7.4, the slices were further incubated with the [3H]-adenine (25 μCi/20 ml) for 45 min. After washing with Krebs buffer, the gravity-packed slices (50 μl) were pipetted into plastic tubes containing Krebs buffer and incubated for 10 min. Then tested compounds were added in 10 μl of the medium, to give a total volume of 500 μl. The incubation was terminated after 10 min with 550 μl of a cool 10% trichloroacetic acid and then the slices were homogenized and centrifuged (10,000 × g, 10 min). [3H]-cAMP was extracted from the supernatant by a sequential Dowex-alumina chromatography. Prior to extraction, samples were spiked with [14C]-cAMP to allow for a percentage recovery correction. The accumulation of cAMP during a 10 min stimulation period was assessed as a percentage of the conversion of [3H]-adenine into [3H]-cAMP.

Statistics

All values are the means of eight rats per experimental group ± SEM. The data were evaluated by one-way analysis of variance (ANOVA) followed by Newman-Keuls multiple comparison test (cAMP accumulation studies) or Student's t-test (binding studies). p < 0.05 was considered significant. Data analysis was performed using Graph Pad Prism (Graph Pad Software, San Diego, CA, USA).

Results

Binding studies

Chronic (21-day) treatment with imipramine did not induce any changes in the density or affinity of radio-
ligand $[^{3}H]$-LY341495 binding to group II mGlu receptors in the rat brain cerebral cortex and hippocampus (Tab. 1).

Measurements of cAMP accumulation

Forskolin (30 µM) induced a robust, ca. 16-fold increase in the cAMP accumulation in rat brain cortical slices vs. basal level. Both forskolin (30 µM)-stimulated and basal cAMP accumulation were not changed by acute or repeated (21 days) imipramine treatment. Group II mGlu receptors agonist, 2R,4R-APDC, inhibited forskolin-stimulated cAMP accumulation in slices from vehicle-treated animals. The similar effect was observed in slices prepared from animals, which received a single injection of imipramine. In contrast, no significant inhibition of forskolin-stimulated cAMP accumulation by 2R,4R-APDC was observed in slices from animals chronically treated with imipramine (Fig. 1).

Discussion

The results of our study show that chronic treatment with an antidepressant drug, imipramine, did not induce any changes in the density of group II mGlu receptors in the rat cerebral cortex or hippocampus. However, cAMP accumulation studies indicated that inhibitory properties of group II mGlu receptors were significantly diminished after repeated imipramine treatment.

The involvement of group II mGlu receptors in the mechanism of action of imipramine has been previously observed by Matrisciano et al. [9], who used Western blot analysis to show that a chronic 21-day treatment with imipramine up-regulated the mGlu2 and mGlu3 receptor expression and function in the hippocampus and several other regions related to depression [9]. Our binding studies did not confirm such observations, since the total density of group II mGlu receptors in the hippocampus and frontal cortex ($B_{\text{max}}$) was not changed after repeated 21-day imipramine administration. However, it must be noticed that two different methods were used to analyze an influence of imipramine on the expression of group II mGlu receptors in the study of Matrisciano and ours.
Immunoblot analysis reflects changes in the level of the total receptor protein, regardless of its functional properties, while binding studies show changes in the total number of binding sites, being not only a quantitative but also qualitative analysis of the changes in the receptor protein. Moreover, different rat strains and treatment schedules may account for the discrepancy between the results obtained from Western blot and binding studies.

In order to analyze an influence of chronic imipramine treatment on the functional activity of group II mGlu receptors we performed cAMP accumulation studies. Group II mGlu receptors are negatively linked to the adenyl cyclase signal transduction pathway; thus, the activation of mGlu2 and mGlu3 receptors induces a decrease in cAMP accumulation [5]. In order to analyze inhibitory properties of a group II mGlu receptor agonist, we used forskolin, which directly activates adenyl cyclase and increases cAMP accumulation in vivo. In our studies, group II mGlu receptor agonist, 2R,4R-APDC, dose-dependently decreased forskolin-induced cAMP formation in cerebral cortical slices of vehicle-treated rats. The similar results were observed in a group of rats, which received a single imipramine injection. In contrast, in a group of rats treated with imipramine for 21 days, we did not observe any significant influence of 2R,4R-APDC on the forskolin-stimulated cAMP formation. It suggests that repeated imipramine administration reduced inhibitory effects related to group II mGlu receptor activation. In other words, it might be supposed that chronic imipramine treatment induces functional attenuation of group II mGlu receptor activity. This finding seems to be consistent with antidepressant-like properties of group II mGlu receptor antagonists in rodents [2, 21].

Our binding studies suggest that the reduction in inhibitory properties of group II mGlu receptors is not related to a possible influence of imipramine on the density of mGlu2 and mGlu3 receptors (Bmax) or affinity of group II mGlu receptor ligands (Kd). However, it is worth mentioning that we observed about 50% higher Kd value in cerebral cortices in four out of eight rats treated chronically with imipramine, compared to the controls, which may suggest a tendency for the affinity of group II mGlu receptor ligands to be increased. On the basis of current knowledge, we suppose that an influence of imipramine on post-receptor targets may be responsible for the decreased reactivity of group II mGlu receptors. A large body of evidence has shown that therapeutic antidepressant effects induced by chronic treatment with antidepressants may be related to the regulation of intracellular signal transduction pathways [3]. For instance, an increased level of Gs protein has been reported in prefrontal cortical samples obtained postmortem from depressed patients who committed suicide. These effects were significantly decreased by antemortem antidepressant treatment [6]. Group II mGlu receptors are linked to adenyl cyclase, which synthesizes cAMP via inhibitory Gs protein [5]. Thus, we suppose that the inhibition of Gs expression and/or function after chronic imipramine treatment may be related to the decreased inhibitory effects of group II mGlu receptors. Furthermore, cAMP transduction pathway has been shown to be up-regulated after prolonged antidepressant therapy [15]. Increased Gsat protein level or facilitated interaction between adenyl cyclase and Gsat protein, have been observed after prolonged antidepressant treatment [4]. However, it must be remembered that such mechanism seems to be brain region specific; e.g. in the nucleus accumbens, inhibition and not activation of CREB produces an antidepressant-like effect [11]. Thus, cAMP activation seems to be regulated by antidepressant therapy. In our studies, we indeed observed a non-significant tendency of forskolin-stimulated cAMP accumulation to be increased in the frontal cortex, after chronic imipramine treatment. A tendency towards promotion of activation of cAMP transduction pathway in the frontal cortex may partially account for the decreased inhibitory effects of an agonist of group II mGlu receptors. Taking together, our results suggest a decreased reactivity of group II mGlu receptors in rat cerebral cortex after prolonged imipramine treatment, which may be implicated in the antidepressant properties of this drug. However, the mechanism of adaptive changes within mGlu2 and mGlu3 receptors after imipramine administration is not explained and requires further studies.

References:


Pharmaceutical Reports, 2007, 59, 525–530

Received: June 14, 2007; in revised form: October 10, 2007.