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Combined administration of PHCCC, a positive allosteric modulator of mGlu4 receptors and ACPT-I, mGlu III receptor agonist evokes antidepressant-like effects in rats

K. Kłak[1], **A. Pałucha**[1], **P. Brański**[1], **M. Sowa**[1], and **A. Pilc**[1,2]

[1] Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland
[2] Institute of Public Health, Collegium Medicum, Jagiellonian University, Kraków, Poland

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Summary. Numerous pharmacological data indicate involvement of glutamate, the major excitatory neurotransmitter in the brain, in the pathophysiology of several neuropsychiatric disorders. It was shown in the preclinical studies that compounds which can reduce the excess of glutamate release (for example group III metabotropic receptors agonists) possess potential therapeutic properties. Thus we focused our interests on (−)-N-phenyl-7-(hydroxyimino) cyclopropa[b]chromen-1a-carboxamide (PHCCC), which is a positive allosteric modulator of mGlu4 receptor. We examined the potential antidepressant-like activity of PHCCC after injection into the brain ventricles alone, or together with (1S,3R,4S)-1-aminocyclo-pentane-1,3,4-tricarboxylic acid (ACPT-I), a nonselective group III mGlu receptor agonist, using the forced swimming test (FST) in rats. We found that ACPT-I induced a dose dependent antidepressant-like effect in FST, which was blocked by an antagonist of group III mGlu receptors (RS)-alpha-cyclopropyl-4-phosphonophenylglycine (CPPG). PHCCC injected intracerebroventricular was not effective, however when the compound was administered together with non-effective dose of ACPT-I, a profound antidepressant-like activity in FST was demonstrated. This effect was reversed by CPPG, group III mGlu receptors antagonist. Results of our studies indicate that a combined administration positive allosteric modulation of mGlu4 receptor and agonists of group III mGlu receptors may be a promising target in the future treatment of depressive disorder.

Keywords: Depression – Antidepressant activity – Group III mGlu receptors – PHCCC – ACPT-I

Introduction

Glutamate receptors mediate most of the excitatory transmission in the central nervous system. Glutamate receptors are divided into two families, the ionotropic and the metabotropic glutamatergic receptors (Nakanishi and Masu, 1994; Nakanishi, 1992). The ionotropic glutamate receptor family is divided into: NMDA, AMPA and kainate receptor-channels. The metabotropic glutamate receptor family is coupled to G-proteins and consists of three groups with a total of eight subtypes named from mGluR1 to mGluR8. This classification was determined by the similarities in coupling mechanisms, molecular structure and homology of sequences and agonist selectivity (Nakanishi and Masu, 1994). Activation of group I (mGluR1 and mGluR5) metabotropic glutamate receptors stimulates phospholipase C and inositol 1,4,5-triphosphate (IP3) formation that leads to release of Ca^{2+} from intracellular stores. Group II (mGluR2 and mGluR3) and type III (mGluR4, mGluR6, mGluR7 and mGluR8) metabotropic glutamate receptors are coupled to adenylate cyclase inhibition via Gi-protein (Conn and Pin, 1997).

Generally, mGlu receptors seem to have evolved as modulatory mechanisms to control CNS excitability. Many disorders of the central nervous system, including psychiatric ones, have been linked to disturbances in the glutamatergic system (Danysz et al., 1996). Drugs that modulate glutamate activity hold promise for treating wide range of disorders, including depression, anxiety and neurodegenerative disorders. Although NMDA antagonists can produce a variety of desirable effects including antidepressant- and anxiolytic-like ones (Skolnick, 1999; Pilc et al., 2002b) they can not be used clinically because of adverse effects (Danysz et al., 1996). Thus, understanding the ways to modulate rather than inhibit neuronal excitability has great therapeutic significance. Current knowledge provides data that antagonists of group I mGlu receptors and agonists of group III mGlu receptors exert antidepressant-like effect in animal tests

and models (Pilc et al., 2002a; Tatarczyńska et al., 2001, 2002) via subtle inhibition of glutamatergic neurotransmission (Schoepp, 2001).

Since it became clear that mGlu4 receptor is amenable to allosteric modulation and is potentiated by N-phenyl-7-hydroxyliminocyclopropa-[b]chromen-1a-carboxamide (PHCCC) (Maj et al., 2003; Marino et al., 2003) we decided to verify the hypothesis that allosteric potentiators of this receptor may provide an antidepressant-like effect in animals models after injections into the brain ventricles.

Materials and methods

The experiments were performed on male Wistar rats (220–250 g). The animals were group-housed on a 12-h light-dark cycle at a room temperature of 19–21°C, with access to food and water ad libitum. Different experimental groups consisted of 7–11 animals. An observer unaware of the treatment performed the experiments. The Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences in Kraków approved all experimental procedures.

The rats were operated on under equithesin anesthesia. Two stainless steel guide cannulae were implanted stereotaxically into the brain ventricles (P 0.8 mm, L 1.5 mm, H 3.8 mm) (Paxinos and Watson, 1986) and were fixed to the skull with stainless steel screws and dental acrylic cement. After the procedure the animals were caged individually. Seven days later, the rats were subjected to behavioral testing. Intracerebroventricular injections of drugs were made using Hamilton micro syringes connected, via polyethylene tubing to two stainless steel needles (0.3 mm o.d.). The needles were lowered 2 mm below the tip of the guide cannula, at the level of the lateral ventricles. Solutions were administered bilaterally over 60 s. The injection needle remained in place for an additional 60 s before it was removed and replaced with a stylet. All compounds were dissolved in sterile saline with the addiction of 0.1 M NaOH; then 0.1 M HCl was used to adjust pH (pH = 7.2). The solutions of group III mGlu receptor agonist ACPT-1 and/or the mGlu4 receptor allosteric modulator PHCCC were injected into the brain ventricles (i.c.v.) in a volume of 2 µl/site 20 min before the test. An antagonist, CPPG, was administered i.c.v. in a volume of 2 µl/site, 10 min before the injection of the agonist and/or the enhancer. Control rats received vehicle according to the same schedule. All experiments were carried out during the light cycle (9 a.m.–2 p.m.). Different groups of animals were used to perform the behavioral procedure.

The antidepressant-like effects of the tested drugs were evaluated according to the method of Porsolt et al. (1978). The rats were placed individually into glass cylinders (40 cm high, 18 cm in diameter) containing 15 cm of water, maintained at 25°C. After 15 min, they were removed to a drying room (30°C) for 30 min. 24 h later the animals were placed again in the cylinder and the total duration of immobility was measured during a 5 min test.

The locomotor activity was measured in photoresistor actometers (40 × 40 × 25 cm), where the animals were placed individually 20 min after the injection of agonists or 30 min after the injection of an antagonist into the brain ventricles. The number of beam breaks was recorded within 30 min of experimental session. The first measurement was performed 5 min after placing the animals into actometers.

The obtained data were analyzed by using a one-way analysis of variance followed by the Dunnett's test. Specific comparison were carried out with the Newman-Keuls multiple post-hoc test using Graph Pad Prism (Graph Pad Software, San Diego, CA, USA). Statistical significance was achieved when the P value was <0.05. Data are expressed as mean ± S.E.M.

(−)-*N*-Phenyl-7-(hydroxyimino) cyclopropa[*b*]chromen-1a-carboxamide (PHCCC) was a gift from Dr Fabrizio Gasparini, Novartis, Basel, Switzerland. (RS)-alpha-cyclopropyl-4-phosphonophenylglycine (CPPG) and (1S,3R,4S)-1-aminocyclo-pentane-1,3,4-tricarboxylic acid (ACPT-I) was purchased from Tocris Cookson Ltd., Bristol).

Results

ACPT-I, a group III mGlu receptors agonist, injected i.c.v. at a dose of 25 nmol/4 µl/rat, induced a dose-dependent antidepressant-like effect in the FST, ($F(3.28) = 28.49$, $P < 0.001$). CPPG, a group III mGlu receptors antagonist, administered i.c.v. (100 nmol/4 µl/rat) 10 min before ACPT-I injection (25 nmol/4 µl/rat), reversed agonist-induced decrease in the immobility time of rats in the forced swimming test (Fig. 1), ($P < 0.001$). ACPT-I was without effect on the locomotor activity of rats (results not shown).

PHCCC, a positive allosteric modulator of mGlu4 receptors, administered alone into the brain ventricles at doses of 25, 50 and 75 nmol/4 µl/rat did not induce an antidepressant-like effect in the FST (Fig. 2). The enhancer administered at a dose of 25 nmol/4ul/rat together with non-effective dose (3 nmol/4 µl/rat) of a group III mGlu receptors agonist, ACPT-1 significantly decreased the immobility time of rats in this test (by 60%), (Fig. 3), ($F(5.66) = 10.82$, $P < 0.0001$). This effect was reversed by CPPG, a group III mGluR antagonist administered i.c.v. at a dose of 100 nmol/4 µl/rat 10 min before ACPT-1 (3 nmol/4 µl/rat) and PHCCC (25 nmol/4 µl/rat) injection (Fig. 3),

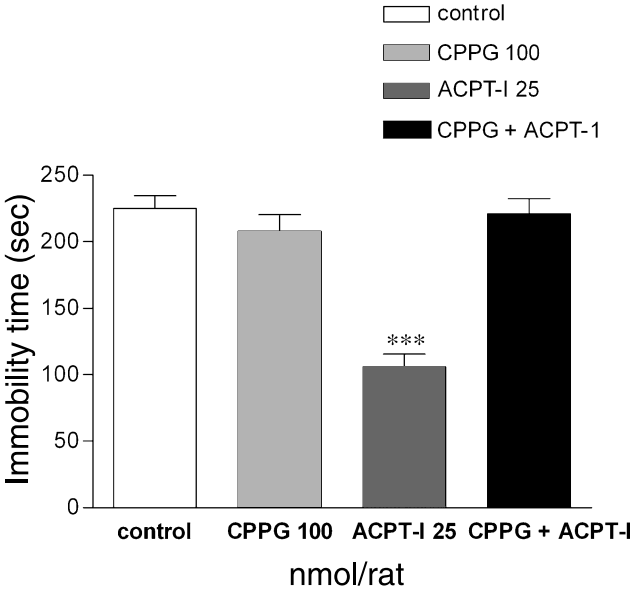

Fig. 1. Effects of ACPT-1 and CPPG in the Forced Swimming Test after administration into brain ventricles. ACPT-1 was injected 20 min before the test and CPPG was injected 10 min before ACPT-1. Values (expressed as means ± S.E.M. (n = 7–9 rats per group) indicate the total immobility time during the 5-min experimental sessions. ANOVA as follows ($F(3.28) = 28.49$); $P < 0.0001$). Symbols indicate significance of differences in Dunnett's Multiple Comparison test. $^{***}P < 0.01$ vs. all other groups

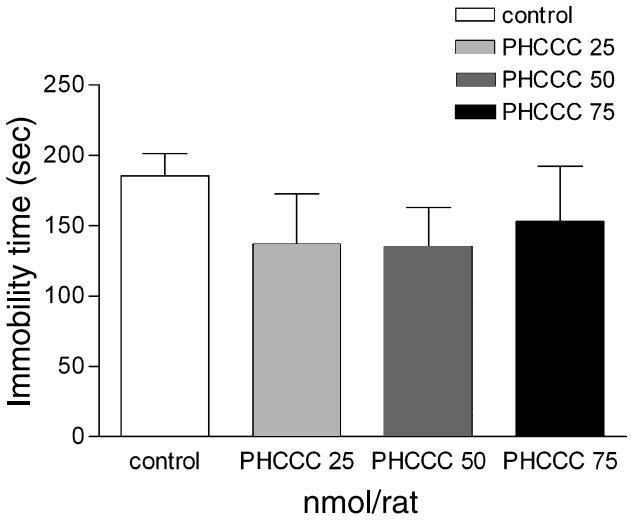

Fig. 2. Effects of PHCCC in the Forced Swimming Test after administration into cerebral ventricles. Drugs were injected 20 min before the test. Values (expressed as means ± S.E.M. (n = 4–7 rats per group) indicate the total immobility time during the 5-min experimental sessions. ANOVA as follows ($F(3.21) = 0.43$; $P > 0.05$)

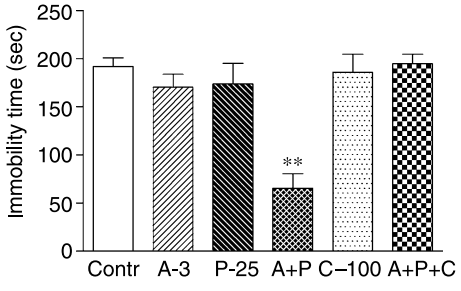

Fig. 3. Effects of PHCCC, ACPT-I and CPPG in the Forced Swimming Test after the administration into cerebral ventricles. ACPT-1 (3 nmol) and PHCCC (25 nmol) were injected individually and/or mixed 20 min before the test. CPPG (100 nmol) was given individually 10 min before the injection of ACPT-1 + PHCCC. Values (expressed as means ± S.E.M. (n = 3–9 rats per group) indicate the total immobility time during the 5-min experimental sessions. ANOVA as follows ($F(5.66) = 10.82$; $P < 0.0001$). A-3 (ACPT-I, 3 nmol), P-25 (PHCCC, 25 nmol), A + P (ACPT-I + PHCCC), C −100 (CPPG, 100 nmol), A + P + C (ACPT-I + PHCCC + CPPG). Symbols indicate significance of differences in Dunnett's Multiple Comparison test $^{**}P < 0.01$ vs. all other groups

Table 1. Effects of ACPT-1 and PHCCC on the locomotor activity in rats

Compounds	Dose (nmol/4 µl)	Number of crossings
Vehicle	0.0	76.5 ± 4.12 (6)
ACPT-1 + PHCCC	3 + 25	60.25 ± 7.54 (7)

The values represent the number of light beams in the actometers within 5 min.

All compounds were administered into the brain ventricles 20 min before the test. Number of rats per group in parentheses. Presented values are the means ± S.E.M.

($P < 0.05$). ACPT-I in combination with PHCCC was without effect on the locomotor activity of rats (Table 1).

Discussion

One of the major needs in the field of depression research is a better understanding of the role of disturbances in excitatory neurotransmission in the process of the disease. The identification of mGlu receptor subtypes is providing new insights into how neuronal excitability is modulated under pathological as well as physiological circumstances (Pałucha and Pilc, 2005). Here we confirm our earlier data that stimulation of group III mGlu receptors by the agonist ACPT-I leads to antidepressant-like effects in the FST (Pałucha et al., 2004).

The availability of a selective positive allosteric modulator of mGlu4 receptors PHCCC (Maj et al., 2003; Marino et al., 2003), which produced anxiolytic-like effects after central administration in rats (Stachowicz et al., 2004), encouraged us to study its possible antidepressant activity. PHCCC, injected intraventricularly was without any effect in the FST in rats. However when injected i.c.v. at a low not effective dose together with ineffective dose of ACPT-1, a non selective group III mGlu receptor agonist, a pronounced antidepressant-like effect was demonstrated in the FST. The antidepressant-like effect was not due to changes (an increase) in locomotor activity, as that parameter was not changed. PHCCC is a close structural analogue of CPCCOEt, both compounds are noncompetitive antagonists of $mGlu_1$ receptors (Maj et al., 2003; Marino et al., 2003) with similar affinities. However, the possible antagonism of $mGlu_1$ receptors is not involved in the antidepressant-like effect of PHCCC, as its effect was blocked by CPPG confirming the involvement of group III mGlu receptors in this interaction. It can be speculated that allosteric potentiation of mGlu4 receptors by PHCCC and stimulation of mGlu7 or mGlu8 receptors by ACPT-I brings about a hyperadditive synergism resulting in antidepressant-like activity.

As group III mGlu receptor agonists are able to depress glutamate release in excitatory synapse in several brain areas including the hippocampus and cerebral cortex (Baskys and Malenka, 1991; Gereau and Conn, 1995; Jin and Daw, 1998), it can be inferred that the mechanism responsible for antidepressant-like activity is due to the decrease in excitatory glutamatergic neurotransmission. Such mechanism may lead to the decreased glutamatergic neurotransmission, an effect similar to that induced by both NMDA and group I mGluR antagonists, which are known to produce antidepressant-like action (for review, see Pałucha and Pilc, 2005; Pilc et al., 2002a; Skolnick, 1999).

It should be emphasized here that many clinically active antidepressants decrease the glutamatergic neurotransmission in the rat cerebral cortex. Such effects were observed both in vivo and in vitro, after acute and/or chronic treatment with several AD, including tricyclic antidepressant drugs, selective serotonin reuptake inhibitors (S SRI) and MAO inhibitors (Gołembiowska and Dziubina, 2000, 2001; Michael-Titus et al., 2000; Bonanno et al., 2005). As in patients with depression glutamate levels are increased (Sanacora et al., 2004) the inhibition of excitatory neurotransmission may be an important effect of antidepressant drugs. Our observations strongly support the hypothesis that the positive allosteric modulation of mGlu4 receptors by means of PHCCC, which potentiates the receptors response induced by ACPT-1 may be a useful therapeutic approach to the treatment of depressive disorder. Moreover, some obstacles like competition with endogenous glutamate or receptor desensitization limit the use of agonists. Discovering the positive allosteric modulators of mGlu receptors gives a possibility to overcome these limitations. To conclude, our studies give a new insight into strategies of the treatment of depressive disorder but this field must be further explored to investigate the optimal and effective compounds to treat this neuropsychiatric disorder.

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Authors' address: Dr. Andrzej Pilc, Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Smetna Street 12, 31-343 Kraków, Poland,
Fax: +4812 6374500, E-mail: nfpilc@cyf-kr.edu.pl