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European Journal of Pharmacology 349 (1998) 83–87

ejp

Antidepressant treatment influences group I of glutamate metabotropic receptors in slices from hippocampal CA1 region

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Received 17 November 1997; revised 19 February 1998; accepted 24 February 1998

Abstract

We investigated the effects of repeated electroconvulsive shock or imipramine treatment on inositol phosphate accumulation and on the reactivity of neurons to metabotropic glutamate (mGlu) receptor agonists in rat hippocampal slices. (1*S*,3*R*)-1-carboxycyclopentane-3-acetic acid (1*S*,3*R*-ACPD), a nonselective mGlu receptor agonist, caused a concentration-dependent increase in inositol phosphate in slices from the CA1 region of the hippocampus, an effect that was not modified by imipramine or electroconvulsive shock treatment. 1*S*,3*R*-ACPD or the selective agonist of the I group of mGlu receptor, (*R*,*S*)-3,5-dihydroxyphenylglycine ((*R*,*S*)-3,5-DHPG), produced a concentration-dependent increase of the population spike recorded in the CA1 cell layer. This effect of 1*S*,3*R*-ACPD was markedly attenuated by both repeated imipramine and electroconvulsive shock treatment, and the action of (*R*,*S*)-3,5-DHPG was markedly attenuated by prolonged imipramine treatment (electroconvulsive shock was not tested). Our results indicate that antidepressant treatment may induce a subsensitivity of group I mGlu receptors when assessed by electrophysiological but not biochemical measures. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Metabotropic glutamate receptor; Imipramine; Electroconvulsive shock; Antidepressant; 1*S*,3*R*-ACPD ((1*S*,3*R*)-1-carboxycyclopentane-3-acetic acid); (*R*,*S*)-3,5-DHPG, ((*R*,*S*)-3,5-dihydroxy phenylglycine); Inositol phosphate; Hippocampus, CA1 region

1. Introduction

Almost all neurotransmitters/neuromodulators have been implicated in the pathophysiology of depression and/or in the mechanism of action of antidepressant drugs, noradrenergic, serotonergic, dopaminergic, cholinergic and γ-aminobutyric acid (GABA-ergic) systems (Janowsky et al., 1972; Lapin and Oxenkrug, 1969; Lloyd et al., 1985; Schildkraut, 1965). Recent data showing the adaptation of NMDA receptor complex after antidepressant treatment (Layer et al., 1995; Skolnick et al., 1996), together with findings that functional NMDA receptor antagonists possess antidepressant-like actions (Layer et al., 1995; Skolnick et al., 1996), indicate the possible involvement of the glutamatergic system in the etiology of depression. Glutamate is abundant in the brain (Mc Geer et al., 1987) and plays a major role in both the physiology and pathophysiology of the central nervous system. Glutamate acts by stimulating ionotropic and metabotropic glutamate receptors (Monaghan et al., 1989; Pin and Duvoisin, 1995).

At least eight subtypes of metabotropic glutamate (mGlu) receptor, (termed $mGlu_1$–$mGlu_8$) have been cloned, divided into three major groups: mGlu I, mGlu II and mGlu III on the basis of sequence similarity, agonist profile and transduction pathways (Pin and Duvoisin, 1995). Receptors from the I group are coupled to phospholipase C (Pin and Duvoisin, 1995), and receptors from the II and III group are coupled to adenylate cyclase. It has been shown that mGlu receptors also regulate neuronal excitability by modulation of several classes of ion channels (Saugstad et al., 1996). The finding that mGlu receptors from the II or the III group are influenced by long-term antidepressant treatment (Pilc and Legutko, 1995a,b) was the reason to investigate whether antidepressive treatments affect also mGlu receptors from the I group. We investigated two different effects exerted by stimulation of group I mGlu receptors: the influence on inositol phosphate accumulation and the influence on the population spikes in the CA1 region of hippocampus after stimulation of the Schaffer collateral– commissural fiber pathway.

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Our results indicate that prolonged electroconvulsive shock or imipramine treatment induces subsensitivity of group I mGlu receptors, as determined by electrophysiological but not by biochemical techniques.

2. Materials and methods

2.1. Animals and housing

Male Wistar rats were kept on a natural light/dark cycle with free access to food and water. Antidepressant drugs were dissolved in water and administered twice daily (at 0800 and 1800) for 14 days (10 mg/kg, 2 ml/kg p.o.; repeated treatment). Electroconvulsive shock treatment consisted of a series of electroshocks (150 mA, 50 Hz, 500 ms) delivered 7 times through ear clip electrodes at 48-h intervals. The controls received saline instead of imipramine or sham electroconvulsive shock (identical handling but no current was passed through the electrodes). Each group consisted of 7–8 animals. The rats were killed 48 h after antidepressant administration in order to avoid possible acute effects of the drugs. All experimental procedures were approved by the IF PAN Animal Care and Use Committee.

2.2. Measurement of inositol phosphate accumulation

The accumulation of inositol phosphate(s) was investigated in slices prepared from the CA1 region of the hippocampus, using a slightly modified version of the method of Kendall and Hill (1990). Briefly, approximately 26 slices (350 μm) cut from each CA 1 region of the hippocampus were further cross-chopped and incubated in 25 ml of gassed ($C0_2$:0_2, 95:5%) Krebs–Henseleit buffer for 40 min, during which time the incubation buffer was changed 3 times. Thirty microliters of gravity-pack slices + 250 μl of Krebs–Henseleit buffer (containing 10 mM LiCl and 1 μCi of 1,2-[^{3}H]*myo*-inositol, 30 Ci/mM, in a volume of 10 μl) were incubated for 45 min at 37°C in a shaking water bath, and then agonists were added in a volume of 10 μl and incubated for another 45 min. Incubations were terminated by addition of 900 μl of acidified chloroform/methanol/10 mM HCl mixture (100:200:1, v/v/v) to each tube together with 10 μl of [^{14}C]inositol-1-phosphate (300 mCi/mM) as a standard. The tubes were vortexed and left on ice for 30 min, after which 310 μl of water and 310 μl of chloroform were added to each tube and the tubes were centrifuged at $2000 \times g$ for 10 min to separate the two phases. A portion (750 μl) of the upper aqueous phase was removed and neutralized with approximately 600 μl of 6.25 mM borax. The samples were pipetted onto Dowex 1-X8 chloride form columns (containing 1 ml of 50% Dowex, washed with 1 M HCl) which were washed with 20 ml of water to remove 1,2-[^{3}H]*myo*-inositol and then inositol phosphates were eluted with 2.5 ml of 1 M HCl into scintillation vials. After addition of 4 ml of Reade Value (Beckman) scintillant the samples were counted in a Beckman liquid scintillation counter.

2.3. Electrophysiological recordings

For electrophysiological studies the hippocampus was cut into 350-μm-thick transverse slices, using a tissue slicer (FHC Brunswick, USA). The slices were transferred to a recording chamber where they were held submerged between two nylon nets and were continuously superfused at a rate of 1.5 ml/min with the medium saturated with 95% O_2 5% CO_2 and maintained at 32°C (pH was 7.4). Electrophysiological recordings were made extracellularly in the CA1 cell layer. Recording microelectrodes were filled with 2 M NaCl (2–4 MΩ). For electrical stimulation (Grass S8 stimulator with an isolation unit), a bipolar, twisted wire electrode was placed in the stratum radiatum to stimulate the Schaffer collateral–commissural fiber pathway. The stimuli were square-wave pulses of 0.1 ms duration, applied at a frequency of 0.1 Hz. The recorded signals were amplified (Axoprobe, Axon Instruments), bandpass-filtered (1 Hz–10 kHz) and stored on a PC hard disk after AD conversion at 5 kHz (a CED interface, Cambridge Electronic). Drugs were administered by superfusion (1 application/slice). The effects of the test substances on the population spike were expressed as percentages of the baseline, pre-drug (control) population spike.

2.4. Analysis of the data

All data in the text are expressed as means ± S.E.M. Statistical analysis was carried out by an analysis of variance (ANOVA) followed by Student's *t*-test or Dunnett test for multiple comparisons.

2.5. Drugs

The drugs used were: [^{14}C]inositol-1-phosphate (300 mCi/mM) and 1,2-[^{3}H]*myo*-inositol, 30 Ci/mM, from American Radiolabeled Chemicals, St. Louis, MO; (1*S*,3*R*)-1-carboxycyclopentane-3-acetic acid ((1*S*,3*R*)-ACPD) and (*R*-*S*)-3,5-dihydroxyphenylgycine (3,5-(*R*,*S*)-3,5-DHPG) from Tocris Cookson, UK; imipramine hydrochloride from Polfa, Poland.

3. Results

The basal accumulation of inositol phosphates was not changed by repeated electroconvulsive shock or imipramine, the values for the control, electroconvulsive shock and imipramine groups were 854 ± 123, 1010 ± 211 and 904 ± 148 dpm, respectively. 1*S*,3*R*-ACPD (Fig. 1) induced a dose-dependent, almost threefold increase in

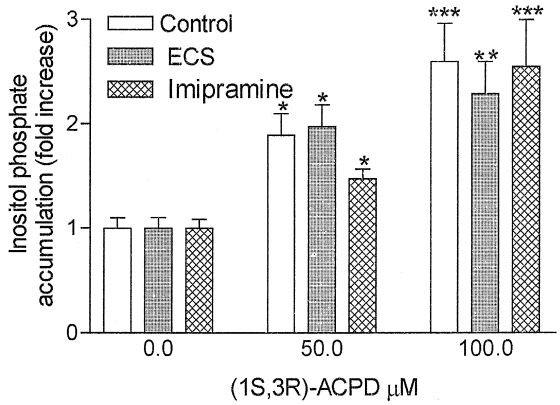

Fig. 1. Effect of imipramine or electroconvulsive shock treatment on the 1*S*,3*R*-ACPD-induced inositol phosphate formation. The data represent the means ± S.E.M. from 6–7 experiments. Assays were performed in triplicate. The basal values for the control, electroconvulsive shock and imipramine groups (marked by 0) were 854 ± 123 dpm, 1010 ± 211 dpm and 904 ± 148 dpm, respectively. $* P < 0.05$, $** P < 0.01$, $*** P < 0.001$ compared to basal accumulation.

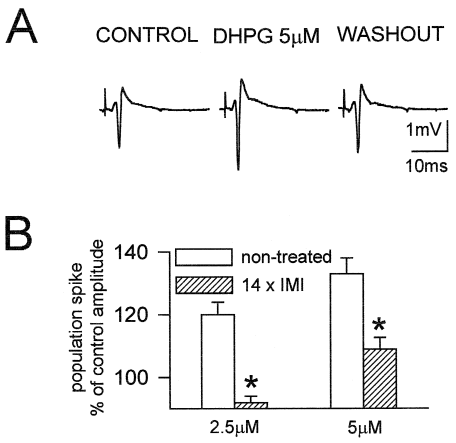

Fig. 3. The influence of (*R*,*S*)-3,5-DHPG on the population spike recorded in the CA1 cell layer. (A) The reversible increase of the population spike elicited by (*R*,*S*)-3,5-DHPG. (B) The effect of prolonged imipramine treatment on the enhancement of the amplitude of population spikes induced by (*R*,*S*)-3,5-DHPG. $* P < 0.05$ vs. non-treated group. The data represent the means ± S.E.M. from 7 animals in each group.

inositol phosphate accumulation in the CA1 region of hippocampus. This action of 1*S*,3*R*-ACPD was not modified significantly by prolonged imipramine or electroconvulsive shock treatment. 1*S*,3*R*-ACPD, a nonselective agonist of mGlu receptors (Pin and Duvoisin, 1995) as well as (*R*,*S*)-3,5-DHPG, a selective agonist of group I mGlu receptors (Schoepp et al., 1994), produced a concentration-dependent increase of the amplitude of the population spikes in the hippocampal CA1 cell layer (Fig. 2A,BFig. 3A,B). These effects were readily reversible upon washout (Fig. 2AFig. 3A). The effects of the lower concentrations of 1*S*,3*R*-ACPD (2.5 μM) or (*R*,*S*)-3,5-DHPG (2.5 μM) on the population spike (Fig. 2B and Fig. 3B) were almost abolished after repeated but not single (not shown) imipramine administration, while the effects of the higher concentrations (5 μM) were significantly attenuated after prolonged imipramine (Fig. 2B and Fig. 3B). The effect of 1*S*,3*R*-ACPD (5 μM) on the population spike was significantly attenuated by repeated (Fig. 2C) but not single (not shown) electroconvulsive shock. The effect of electroconvulsive shock on the action of (*R*,*S*)-3,5-DHPG was not tested.

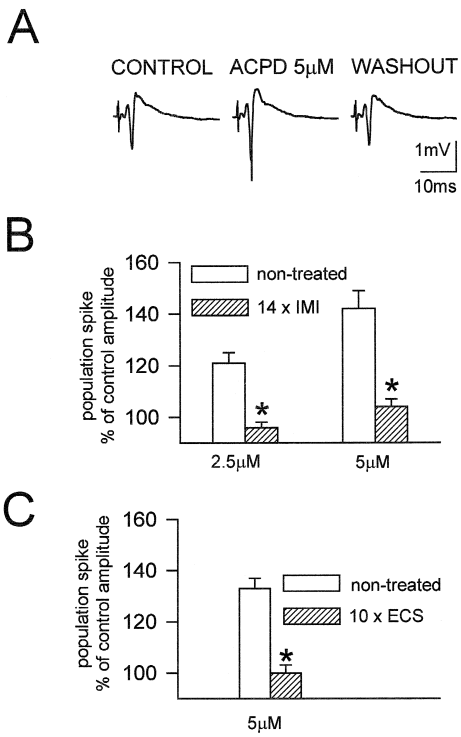

Fig. 2. The influence of 1*S*,3*R*-ACPD on the population spike recorded in the CA1 cell layer. (A) The reversible increase of the population spike produced by 1*S*,3*R*-ACPD. (B) The effect of prolonged imipramine treatment on the enhancement of the amplitude of population spikes induced by 1*S*,3*R*-ACPD. $* P < 0.05$ vs. non-treated group. (C) The effect of prolonged electroconvulsive shock treatment on the enhancement of the amplitude of population spikes induced by 1*S*,3*R*-ACPD. $* P < 0.05$ vs. non-treated group. The data represent the means ± S.E.M. from 7 animals/group.

4. Discussion

Recent studies demonstrate that antidepressant treatment influences glutamatergic receptors in the brain, including the NMDA receptor complex (Skolnick et al., 1996) as well as certain subtypes of the mGlu receptor coupled to adenylate cyclase (Pilc and Legutko, 1995a,b). Here, we show that antidepressant treatment also modifies the sensitivity of group I mGlu receptors. While the sensitivity of mGlu receptors coupled to inositol phosphate production in the CA1 region of hippocampus was not changed, subsensitivity developed toward the excitatory effects of mGlu I receptor agonists in the CA1 region of hippocampus, as determined by measuring of extracellularly recorded population spikes.

The mGlu receptors of the I group are coupled to phospholipase C pathways (Pin and Duvoisin, 1995) through activation of G proteins, leading to the production of two second messengers—diacylglycerol and inositol trisphosphate (IP_3). Therefore, the concentration-dependent increase in inositol phosphate accumulation observed after (1*S*,3*R*)-ACPD in the slices from the CA1 region of the hippocampus can be attributed to the stimulation of the group I mGlu receptors in that structure. The effect of (1*S*,3*R*)-ACPD on inositol phosphate accumulation was not influenced significantly by electroconvulsive shock or imipramine treatment.

It has been shown that activation of the group I mGlu receptors in the CA1 region of hippocampus is responsible for the depolarization of pyramidal neurons (Davies et al., 1995), probably as a result of inhibition of potassium channels (Saugstad et al., 1996). Depolarization and inhibition of Ca^{2+}-activated K^+ currents may contribute to the increase in the amplitude of the population spike evoked by (1*S*,3*R*)-ACPD and (*R*,*S*)-3,5-DHPG in our experiments. Similar effects exerted both by a nonselective agonist of mGlu receptors, (1*S*,3*R*)-ACPD, and a selective agonist of group I mGlu receptors, (*R*,*S*)-3,5-DHPG (Schoepp et al., 1994), indicate that this group of mGlu receptors is involved in the increase of the population spikes observed in our experiments. This increase was markedly attenuated by both repeated imipramine and electroconvulsive shock administration. Since the effect was observed 48 h after chronic drug treatment, when imipramine is not detected in the brain (Daniel et al., 1981) and was not observed after single imipramine injection, it can be attributed to the long-term effects of antidepressant treatment.

Within the group I mGlu receptors, two major subtypes and several splice variants exist (Pin and Duvoisin, 1995). The dissociation between the effects of antidepressants exerted on biochemical and electrophysiological responses mediated by the stimulation of the group I mGlu receptors might indicate that different subtypes of this group of mGlu receptors are responsible for both effects. It can be speculated that the attenuation of the effects of mGlu receptor agonists on population spikes by antidepressant treatment may be due to subsensitivity of the subtype of group I mGlu receptors which is coupled to potassium channels. The sensitivity of the subtype of group I mGlu receptors responsible for an increase in inositol phosphate accumulation is not influenced by antidepressant treatment. However, the lack of subtype-specific agonists and/or antagonists does not allow for this hypotheses to be tested at present. The differences in (1*S*,3*R*)-ACPD concentrations used for the electrophysiological and biochemical studies may also account for the discrepancy between the biochemical and electrophysiological results. Current methodology does not allow us to test similar agonist concentrations, as at low doses (1*S*,3*R*)-ACPD does not produce significant changes in inositol phosphate accumulation and at high doses secondary spikes appear (results not shown), which complicates evaluation of electrophysiological data.

It is of interest that antidepressant treatment induced subsensitivity of CA1 cells to activation of other receptors (β-adrenoceptors, $5\text{-}HT_4$) which inhibit K^+ conductances via intracellular second messengers (Bijak, 1989; Bijak et al., 1997). Such an effect may suggest changes in postreceptor transduction signals evoked by antidepressant treatment. There are several data indicating that G proteins (Lesch et al., 1992) and/or intracellular effectors such as protein kinases, including protein kinase C (Mann et al., 1995), are modified by antidepressant drugs.

Stimulation of group I mGlu receptors, which are coupled via $G_{q/11}$ proteins to inwardly rectifying K^+ channels (Abdul Ghani et al., 1996; Sharon et al., 1997), leads to inhibition of the function of these potassium channels. A decrease in the G protein observed after multiple administration of antidepressant drugs (Lesch et al., 1992) may lead to the observed attenuation of the (*R*,*S*)-3,5-DHPG- and (1*S*,3*R*)-ACPD-mediated increases in the population spikes. Coupling of different G proteins to K^+ channels and to the phospholipase C system, together with differential effects of antidepressants on these proteins, may explain the variability observed in both types of experiments; however, this remains to be tested.

Protein kinase C is also modified by antidepressant treatment (Mann et al., 1995). Since it can modulate K^+ channels, leading to inhibition of their function (Henry et al., 1996; Peretz et al., 1996), the decrease in protein kinase C activity observed after antidepressant treatment (Mann et al., 1995) may contribute to the attenuation of population spikes induced by stimulation of group I mGlu receptors by antidepressants.

Our results indicate that the sensitivity of the hippocampal group I mGlu receptors whose activation leads to inositol trisphosphate accumulation was not changed by imipramine or electroconvulsive shock. The responsiveness of the group I mGlu receptors whose stimulation leads to an increase in the amplitude of population spike recorded in the CA1 cell layer was attenuated by both treatments. The dissociation between the electrophysiological and biochemical responses elicited by stimulation of mGlu receptors may be either due to the involvement of different subtypes of receptors in both responses or due to the postreceptor effects of antidepressant drugs on G proteins and/or protein kinase C. Our results suggest that antidepressive therapy alters the responsiveness of hippocampal neurons to group I mGlu receptor agonists. It is possible that this modification may play a role in the mechanism of action of antidepressants.

Acknowledgements

This project was supported by the Institute of Pharmacology, Polish Academy of Sciences.

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