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REPEATED ADMINISTRATION OF IMIPRAMINE ATTENUATES GLUTAMATERGIC TRANSMISSION IN RAT FRONTAL CORTEX

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Abstract—The effects of repeated administration of a tricyclic antidepressant, imipramine, lasting 14 days (10 mg/kg p.o., twice daily), were studied ex vivo in rat frontal cortex slices prepared 48 h after last dose of the drug. In slices prepared from imipramine-treated animals the mean frequency, and to a lesser degree the mean amplitude, of spontaneous excitatory postsynaptic currents recorded from layer II/III pyramidal neurons, were decreased. These effects were accompanied by a reduction of the initial slope ratio of pharmacologically isolated *N*-methyl-D-aspartate to AMPA/kainate receptor-mediated stimulation-evoked excitatory postsynaptic currents. Imipramine treatment also resulted in a decrease of extracellular field potentials evoked in layer II/III by stimulation of underlying sites in layer V. These results indicate that chronic treatment with imipramine results in an attenuation of the release of glutamate and an alteration in the postsynaptic reactivity of ionotropic glutamate receptors in rat cerebral cortex.

Key words: tricyclic antidepressant, neocortex, brain slices, AMPA/kainate receptors, NMDA receptors.

Growing evidence indicates that abnormalities in the excitatory amino acid transmission play an important role in the pathophysiology of mood disorders and that a common mechanism of various antidepressant therapies may involve modifications in the function of the glutamatergic system (reviewed in: Paul and Skolnick, 2003; Kugaya and Sanacora, 2005; Palucha and Pilc, 2005; Schechter et al., 2005). Recent work has implicated dysregulation of AMPA and *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic transmission in depression (Bleakman et al., 2007; Pittenger et al., 2007). The effectiveness of the inhibition of glutamate release by lamotrigine and riluzole has been demonstrated in the treatment of certain mood disorders (reviewed in: Carlson et al., 2006). To achieve a therapeutic effect, repetitive administration of antidepressant drugs for at least 2–3 weeks is necessary. This phenomenon has been linked to a slow time course of the development of adaptive modifications in several brain neurotransmitter systems, but mechanisms of these modifications remain incompletely understood (reviewed in: Holtzheimer and Nemeroff, 2006). One of human brain structures, where both structural and functional abnormalities have been found to occur in course of mood disorders, is the frontal cortex (Drevets, 2001).

In frontal cortex of rats, chronic treatment with a number of antidepressant drugs, including a tricyclic antidepressant imipramine, reduces radioligand binding to NMDA receptors (Nowak et al., 1993, 1996; Skolnick et al., 1996). Chronic treatment of mice with imipramine induces region-specific effects on the level of mRNAs encoding NMDA receptor subunits (Boyer et al., 1998). NMDA receptor antagonists exhibit antidepressant-like actions in animal models and potentiate the effects of antidepressants (Trullas and Skolnick, 1990; Maj et al., 1992; Petrie et al., 2000). It has been hypothesized that the mechanism of antidepressant action involves dampening of the function of NMDA receptors (reviewed in: Skolnick, 1999), however, more recent work has demonstrated that antidepressant treatment results also in an increased expression of AMPA receptors in rat hippocampus (Martinez-Turrillas et al., 2002). Moreover, a decreased potassium-stimulated glutamate outflow, consistent with a reduced release of synaptic glutamate, has been reported to occur in rat prefrontal cortex after imipramine treatment (Michael-Titus et al., 2000).

We have previously shown that repeated administration of imipramine or citalopram, lasting 2 weeks, resulted in a decrease in the amplitude of glutamate-mediated field potentials evoked in layer II/III of rat frontal cortex by stimulation of underlying sites as well as in a reduction in the amplitude ratio of pharmacologically isolated NMDA to AMPA/kainate receptor-mediated components of the field potential (Bobula et al., 2003). These results suggested that chronic treatment with antidepressants may attenuate glutamatergic transmission in the cerebral cortex. However, the extracellular recording technique does not allow for investigating the mechanism of this effect in more detail. Therefore, in the present study we aimed at finding the effects of repeated administration of imipramine on glutamatergic transmission in rat frontal cortex using whole-cell recording.

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Abbreviations: aCSF, artificial cerebrospinal fluid; CGP 37849, (±)-2-amino-4-methyl-5-phosphono-3-pentenoic acid; eEPSC, evoked excitatory postsynaptic current; NBQX, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide; NMDA, *N*-methyl-D-aspartate; sEPSC, spontaneous excitatory postsynaptic current; TTX, tetrodotoxin.

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EXPERIMENTAL PROCEDURES

Animals

Experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences, and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) guidelines for the use of experimental animals and national law. All efforts were made to minimize the number of animals used and their suffering. Male Wistar rats, weighing approx. 120 g at the beginning of the experiment, were obtained from the animal facility of the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland. Animals were housed in groups and maintained on a 12-h light/dark schedule with standard food and tap water *ad libitum*.

Imipramine treatment and slice preparation

Imipramine (Polfa, Poland), dissolved in water, was administered per os (dose: 10 mg/kg, volume: 2 ml/kg) twice daily, for 14 days (Bobula et al., 2003; Zahorodna et al., 2006). Earlier work has shown that this dose and this treatment schedule induce adaptive changes in brain 5-HT and dopamine receptors as well as alter the behavioral reactivity of the central 5-HT, dopamine and adrenergic systems in rats (Maj et al., 1996, 1998; Rogóz, 2007). Control rats received water but otherwise they were handled identically and were investigated concurrently with imipramine-treated animals.

Brain slices were prepared 2 days after the last imipramine administration. The intracerebral half-life of imipramine is 2.4±0.3 h (Sato et al., 1994). Rats were decapitated, their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 130 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.25 KH_2PO_4, 26 $NaHCO_3$, 10 D-glucose and bubbled with the mixture of 95% O_2/5% CO_2. Frontal cortical slices (400 μm thick) were cut in the coronal plane using a vibrating microtome (VT1000; Leica Microsystems, Wetzlar, Germany). Slices were stored submerged in aCSF at 32±0.5 °C.

Field potential recording

A slice was superfused in the recording chamber of the submerged type at 2.5 ml/min with warm (32±0.5 °C), modified aCSF of the following composition (in mM): 132 NaCl, 2 KCl, 1.25 KH_2PO_4, 26 $NaHCO_3$, 1.3 $MgSO_4$, 2.5 $CaCl_2$, and 10 D-glucose 10, bubbled with 95%O_2/5%CO_2 (Bobula et al., 2003). A bipolar stimulating electrode (FHC, USA) was placed approx. 2 mm lateral to the midline and approx. 1.5 mm below the pial surface (layer V). Constant-voltage stimuli (0.2 ms, 4–15 V) were delivered at 0.1 Hz. Field potentials were recorded using glass micropipettes filled with 2 M NaCl (2–5 MΩ), which were placed approx. 0.3 mm below the cortical surface (layer II/III). The contribution of antidromic activation was diminished by a slightly off-radial (approx. 50 μm) placement of the recording and stimulating electrodes. Recordings were amplified (Axoprobe, Axon Instruments, USA), bandpass filtered (1–500 Hz), acquired on a PC computer using 1401 interface (CED, UK) and analyzed on- and off-line using SIGAVG software (CED). The initial slope of the field potential was measured over 1 ms at a fixed latency from the stimulus. Stimulus-response curves obtained for each slice were fit with the Boltzmann equation: $A_i = A_{max}/(1+\exp((u-u_h)/-b))$; where: A_{max}, maximum initial slope; u, stimulation intensity; u_h, the stimulation intensity which evoked field potential of half-maximum initial slope; b, factor proportional to the slope of the curve (Zahorodna et al., 2006).

Whole-cell recording

A slice was placed in the recording chamber where it was submerged and superfused at 3 ml/min with warm (32±0.5 °C), modified aCSF of the composition identical to that used in field potential experiments. Neurons were visualized using Zeiss Axioskop (Zeiss, Oberkochen, Germany) upright microscope using Nomarski optics, a 40× water immersion lens and an infrared camera (Tokarski et al., 2003). Layer II/III neurons were sampled from sites located approx. 2 mm lateral to the midline and approx. 0.3 mm below the pial surface. Patch pipettes were pulled from borosilicate glass capillaries (Clark Electromedical Instruments, Kent, UK) using Sutter Instrument (Novato, CA, USA) P87 puller. The pipette solution contained (in mM): 130 K-gluconate, 5 NaCl, 0.3 $CaCl_2$, 2 $MgCl_2$, 10 Hepes, 5 Na_2-ATP, 0.4 Na-GTP, and 1 EGTA. Osmolarity and pH were adjusted to 290 mOsm and 7.2, respectively. Pipettes had open tip resistance of approx. 6 MΩ. Signals were recorded using the SEC 05LX amplifier (NPI, Germany), filtered at 2 kHz and digitized at 20 kHz using Digidata 1322A interface and Clampex 9.2 software (Molecular Devices, USA). Pyramidal cells were identified by the shape of the soma and the presence of a prominent apical dendrite (Fig. 2a) as well as the regular spiking pattern showing adaptation in response to a depolarizing current pulse (Fig. 2b).

Analysis of spontaneous excitatory postsynaptic currents (sEPSCs) and evoked excitatory postsynaptic currents (eEPSCs)

After confirming the characteristics of the neuron in the current clamp mode, cells were voltage-clamped at −76 mV for a period of 8 min. Data were accepted for analysis when the access resistance ranged between 5 and 8 MΩ and it was stable (<25% change) during recording. Spontaneous EPSCs were detected off-line and analyzed using Mini Analysis software (Synaptosoft, USA). Since the noise level of recordings was in a range of 4–5 pA, amplitude and area thresholds for the detection of an event were set to 7 pA and 25 fC, respectively, which maximized correct identification of sEPSCs. Recorded traces were visually inspected following automated analysis to prevent false positive identification and false negative rejection of events.

Postsynaptic currents were evoked with a bipolar stimulating electrode positioned at the depth corresponding to layer V (approx. 1.5 mm below the pial surface and approx. 50 μm off-radial as in field potential recording). Stimulus intensity (duration: 0.1 ms, frequency: 0.033 Hz) was adjusted to evoke a response with an average amplitude of approx. 150 pA in neurons held at −76 mV. For recording of AMPA/kainate receptor-mediated eEPSC, slice was perfused with aCSF containing 2 μM (±)-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 37849, Tocris Bioscience, Bristol, UK), an NMDA receptor antagonist. For each cell 20 successive responses were averaged. Then, for recording of NMDA receptor-mediated eEPSCs, after washout of CGP 37849, aCSF devoid of Mg^{2+} ions and containing 5 μM 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX, disodium salt, Tocris), a specific AMPA/kainate receptor antagonist, was introduced (Bobula et al., 2003). After the stabilization of responses 20 traces were averaged. The application of aCSF containing CGP 378499 and NBQX lasted for 20 min in each case. To minimize the contribution of $GABA_A$ receptors to recorded responses, initial eEPSC slope was measured at a fixed latency from the stimulus over 1–2 ms (Brager and Thompson, 2003). In some of the experiments bicuculline methiodide (Tocris) was added to the aCSF to block $GABA_A$ receptors and tetrodotoxin (TTX, Sigma-Aldrich, Poznan, Poland) to block Na^+ channels.

Statistics

Results are expressed as means±S.E.M. Statistical analysis was carried out using Student's *t*-test or Mann-Whitney *U* test, where indicated. Cumulative histograms were constructed using Clampfit 9.2 software (Molecular Devices, USA).