Behavioural Brain Research 317 (2017) 319–326

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Imipramine ameliorates early life stress-induced alterations in synaptic plasticity in the rat lateral amygdala

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HIGHLIGHTS

- Maternal separation stress reduced both LTP and LTD at the thalamic input to the lateral amygdala.
- Maternal separation stress impaired LTP and enhanced LTD at the cortical input to the lateral amygdala.
- Treatment with imipramine restored the extent of the synaptic modification range at the thalamic input.
- Treatment with imipramine restored the potential for LTD, but not for LTD, at the cortical input.
- Imipramine-mediated restoration of synaptic plasticity will have consequences for amygdala-dependent learning.

ARTICLE INFO

Article history:
Received 28 July 2016
Received in revised form
27 September 2016
Accepted 28 September 2016
Available online 29 September 2016

Keywords:
Long-term potentiation
Long-term depression
Field potentials
Maternal separation
Brain slices

ABSTRACT

Long-term potentiation (LTP) and long-term depression (LTD) are two opposite forms of synaptic plasticity at the cortical and thalamic inputs to the lateral amygdala (LA). It has been demonstrated that maternal separation (MS) of rat pups results in alterations in the potential for both pathways to undergo LTP and LTD in adolescence. Imipramine, a prototypic tricyclic antidepressant, has been shown to counteract some detrimental effects of MS on rat behavior, however it is not known whether MS-induced alterations in the potential for bidirectional synaptic plasticity in the LA could be reversed by imipramine treatment. To this end, rat pups were subjected to MS (3 h/day) on postnatal days (PNDs) 1–21. On each of PNDs 29–42, male rats previously subjected to MS were injected subcutaneously with imipramine (10 mg/kg). Field potentials were recorded *ex vivo* from slices containing the LA and saturating levels of LTP and LTD were induced. At the thalamic input to the LA, both the maximum LTP and the maximum LTD were reduced in rats subjected to MS when compared to control animals, confirming earlier results. However, these effects were no longer present in rats subjected to MS and later treated with imipramine. At the cortical input in slices prepared from MS-subjected rats, an impairment of the maximum LTP and an enhancement of the maximum LTD were observed. At the cortical input in rats subjected to MS and receiving imipramine treatment, the level of LTD was comparable to control but imipramine did not restore the potential for LTP at this input. These results demonstrate that imipramine fully reverses the effects of MS in the thalamo-amygdalar pathway, however, in the cortico-amygdalar pathway the reversal of the effects of MS by imipramine is partial.

1. Introduction

Maternal separation (MS) of rat pups, a commonly used model of early life stress, affects growth and development of the brain and results in the occurrence of anxiety-like and depressive behaviors during adulthood [reviewed in [1]]. It has been shown that imipramine, a prototypic tricyclic antidepressant of the first generation, which is still regarded effective for treatment of depression in human patients [reviewed in [2]], ameliorates MS-induced increases in the immobility time of rats subjected to the forced

Abbreviations: ACSF, artificial cerebrospinal fluid; FP, field potential; IMI, imipramine; LA, lateral amygdala; LFS, low frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; MS, maternal separation; PND, postnatal day; TBS, theta burst stimulation.

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http://dx.doi.org/10.1016/j.bbr.2016.09.065
0166-4328/

swim test as a model of depressive-like behavior [3]. Imipramine acts primarily as an inhibitor of serotonin and norepinephrine reuptake [reviewed in [4,5]], however, administration of this drug has been shown to induce a spectrum of other effects in the rat brain, including profound changes in the expression pattern of numerous genes [6]. Recent work has shown that imipramine influences the activity of protein kinases involved in promoting synaptic plasticity [7], reverses stress-induced social avoidance behavior by blocking neuroinflammatory signaling [8] and reverses the effects induced by chronic mild stress on behavior including impaired recognition memory, decreased social interactions and anxiety [9]. Imipramine administration attenuates glutamate release from presynaptic terminals and reduces the reactivity of postsynaptic NMDA receptors in the frontal cortex [10–12]. Moreover, imipramine counteracts the enhancement of glutamatergic transmission and impairment of long-term potentiation (LTP), which occur in the frontal cortex of rats receiving repeated corticosterone injections as a model of subchronic stress [13].

MS stress has been reported to modify the conditioned fear reaction [14–16]. We have previously demonstrated that MS alters the synaptic modification range at the cortical and thalamic inputs to the lateral amygdala (LA) [17], a key structure involved in fear conditioning [reviewed in [18,19]]. Since imipramine has been shown to counteract some of MS-related behavioral alterations in adult rats, in the present study we aimed to determine whether MS-induced alterations in the synaptic modification range in the rat LA could be ameliorated by repeated imipramine administration lasting for two weeks.

2. Materials and methods

2.1. Animals and treatment

The experimental protocols were approved by the local Animal Care and Use Committee at the Jagiellonian University 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.

Wistar dams were housed together with their offspring in standard light/darkness conditions (light on: 7.00–19.00) with food and water available *ad libitum*. On each of postnatal days (PNDs) 1–21 the dams were removed from the maternity cages. They were kept individually for 3 h in holding cages and then returned to maternity cages. During that time the litter stayed in home cages. Young animals were weaned at PND 28 and housed in groups (4–6 rats per cage). On each of PNDs 29–42 male rats previously subjected to MS and animals from the control, unstressed group, were injected subcutaneously with imipramine (Sigma; dose: 10 mg/kg) dissolved in 0.9% NaCl (termed: saline; volume: 2 ml/kg). Another group of MS-rats and a group of control (unstressed) male rats received injections of saline (volume: 2 ml/kg) on PNDs 29–42.

2.2. Slice preparation

Between PND 43–PND 60 the rats were anesthetized with isoflurane and decapitated. Their brains were removed from the skull and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 4 $MgSO_4 \cdot 7 H_2O$, 4.5 KCl, 2 $CaCl_2$ and 10 D-glucose, bubbled with a mixture of 95% O_2 and 5% CO_2. The brains were then cut into slices (450 μm thick) in the coronal plane using a vibrating microtome (VT 1000 Leica Microsystems, Germany). Slices containing the lateral nucleus of the amygdala (LA) were transferred to an interface-type recording chamber and perfused at 2 ml/min with a modified ACSF ($33.5 \pm 0.2\,^{\circ}C$) in which the concentration of $MgSO_4$ was lowered to 1 mM.

2.3. Field potential recording

Recordings began approx. 2.5 h after slice preparation. Field potentials (FPs) were evoked by stimulating (0.05 Hz, pulse duration: 100 μs) the external or internal capsule to activate cortico-amygdalar or thalamo-amygdalar connections respectively [20]. FPs were recorded using ACSF-filled glass micropipettes (1–3 MΩ). Signals were amplified (Axoprobe 2, Axon Instruments, USA), band-pass filtered (1 Hz–5 kHz) and A/D converted (micro1401 interface, Signal 2 software, CED, UK). After measurement of the relationship between the stimulus intensity and amplitude of the early, negative-going wave of FPs (input-output curve) stimulation intensity was adjusted to evoke FPs of 50% of maximum response. Next, after 15 min of baseline recording LTP was induced by theta burst stimulation (TBS) composed of six trains of pulses delivered at 5 Hz. Each train consisted of eight pulses delivered at 100 Hz. TBS was repeated three times every 5 min. To reach the saturation level of LTP, TBS sequences were repeated 4 and 5 times at the thalamic and the cortical input to the LA, respectively, every 45 minutes [17]. LTD was induced by low frequency stimulation (LFS, 900 pulses at 1 Hz). In both inputs LFS sequences were delivered 5 times to saturate LTD.

2.4. Data analysis

Statistical analyses were performed using the SigmaPlot 11.0 software (Systat Software, Inc.). The mean amplitude of responses recorded after the last conditioning stimulation was compared to the initial baseline values and expressed as a percentage of change. Group comparisons employed two-way analysis of variance (ANOVA) with the type of treatment (MS or control) as the first factor and the drug administered (imipramine or saline) as the second factor. Two-way ANOVA was followed by the Tukey's post-hoc test and $p < 0.05$ was taken as significant. Data are presented as group means ± SEM.

Stimulus-response plots were fitted with the Boltzmann equation: $V_i = V_{max}/(1 + \exp((u\text{-}u_h)/\text{-}S))$. The following parameters were compared: the maximum field potential amplitude (V_{max}), the stimulation intensity evoking a field potential of half-maximum amplitude (u_h) and the factor proportional to the slope of the curve (S).

3. Results

3.1. The effects of maternal separation and imipramine on basal synaptic transmission in the LA

Analyses of field potentials evoked in the LA by the stimulation of the thalamo-amygdalar pathway revealed MS-related reduction of FPs over a wide range of stimulation intensities compared to slices originating from control animals receiving either imipramine or the vehicle (Table 1; Fig. 1A_1). This effect of MS was absent in preparations originating from MS rats receiving imipramine treatment (Fig. 1A_2). In contrast, in the cortico-amygdalar pathway no differences in FPs were detected between slices obtained from control and MS rats receiving either imipramine or saline (Table 2; Figs. 1B_1, B_2).

3.2. Imipramine treatment reverses the effects of maternal separation on synaptic plasticity at the thalamic input to the LA

In slices obtained from MS-subjected rats we observed a reduction of LTP measured after the last TBS episode (136 ± 7% of