Neuroscience 232 (2013) 161–168

ACUTE ANTIDEPRESSANT TREATMENT DIFFERENTLY MODULATES ERK/MAPK ACTIVATION IN NEURONS AND ASTROCYTES OF THE ADULT MOUSE PREFRONTAL CORTEX

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Abstract—The onset of action of antidepressants (ADs) usually takes several weeks, but first molecular responses to these drugs may appear already after acute administration. The Extracellular Signal-regulated Kinase/Mitogen-Activated Protein Kinase (ERK/MAPK) signaling pathway is a target of ADs and an important pathway involved in cellular plasticity. In major depressive disorder (MDD), especially the prefrontal cortex (PFC) and hippocampus (Hip) are most likely affected in depressive patients and recent work revealed a hyperactivated ERK signaling in the rat PFC after chronic stress, a precipitating factor for MDD. Strong evidences support that not only neurons but also astrocytes participate in neuronal activity and may therefore additionally be a substrate of AD action. In this study, we show by Western blot that neither fluoxetine (FLX) nor desipramine (DMI) preferentially affects the activation of one of the two ERK isoforms, ERK1 and ERK2, with respect to the other. Further immunohistochemical analysis in the PFC revealed that basal levels of phospho-activated ERK (pERK) are mostly found in neurons in contrast to very few astrocytes. Both ADs can inhibit neuronal pERK as early as 15 min after drug administration with peculiar regional and layer specificities. Contrarily, at this time point none of the two ADs shows a clear modulation of astrocytic pERK. We propose that this mechanism of action of ADs may be protective against an exacerbated cortical ERK activity that may exert detrimental effects on susceptible neuronal populations. Our findings on acute effects of AD treatment in the adult mouse PFC encourage to examine further how this treatment might influence pERK in animal models of depression to identify early targets of AD action.

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Abbreviations: ADs, antidepressants; ANOVA, analysis of variance; CLSM, confocal laser scanning microscopy; CPu, caudato-putamen; DMI, desipramine; FLX, fluoxetine; GFAP, glial fibrillary acidic protein; Hip, hippocampus; IF–IHC, immunofluorescent–immunohistochemistry; i.p., intraperitoneal; MAPK, Mitogen-Activated Protein Kinase; MDD, major depressive disorder; pERK, phospho-activated ERK; PFC, prefrontal cortex; RT, room temperature.

Key words: antidepressants, neuron, astrocyte, mouse, prefrontal cortex, ERK.

INTRODUCTION

The Extracellular Signal-regulated Kinases ERK1 and ERK2 belong to the Mitogen-Activated Protein Kinase (MAPK) signaling pathway, a well-known family of protein kinases that play a role in various cellular processes such as proliferation, differentiation, survival and apoptosis (Kolch, 2000; Avruch, 2007; Tanis and Duman, 2007; Lefloch et al., 2009; Voisin et al., 2010). Because of the high similarity of their sequences, it has long been a matter for debate whether ERK1 or ERK2 is functionally redundant or they execute separated functions (Vantaggiato et al., 2006; Lefloch et al., 2009).

In the last years, a relevant implication of the ERK signaling pathway in major depressive disorder (MDD) has been highlighted (Dwivedi et al., 2001; Einat et al., 2003; Qi et al., 2009; Duric et al., 2010). Moreover, activation of ERK1/2 has been suggested to be important for synaptic remodeling and plasticity (Atkins et al., 1998; Patterson and Yasuda, 2011). These functions are impaired in animal models of depression and antidepressants (ADs) have been shown to restore neuronal networks through modulation of structural and synaptic plasticity (Qi et al., 2008; Bessa et al., 2009); thereby, strongly suggesting the ERK1/2 pathway as a substrate that contributes to the therapeutic efficacy of ADs (Fumagalli et al., 2005; Tanis and Duman, 2007). Although the relief of clinical symptoms of depression requires several weeks of AD treatment before beneficial effects become manifest, the first cellular responses that induce long-term plasticity changes can occur already much earlier. In support of these early changes with late effects, we have shown *in vitro* that ADs can activate the ERK/MAPK signaling pathway in C6 glioma cells with a peak of activation 10 min after AD administration that is sufficient to induce an increased release of glia cell-derived neurotrophic factor (GDNF) 48 h later (Di Benedetto et al., 2012).

Among all brain regions investigated for their role in controlling emotional states and mood disorders, especially the prefrontal cortex (PFC) and hippocampus (Hip) have been repeatedly described to be affected in MDD patients (Einat et al., 2003; Fumagalli et al., 2005; Qi et al., 2008). Specifically, a hyperactivated ERK signaling has been observed in neurons of the rat PFC

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http://dx.doi.org/10.1016/j.neuroscience.2012.11.061

after chronic stress, a precipitating factor for depression (Trentani et al., 2002; Kuipers et al., 2003; Gerrits et al., 2006). Moreover, a very recent study pointed out that a specific subpopulation of corticostriatal projection neurons in layer V of the cortex is essential for the effects of fluoxetine (FLX) (Schmidt et al., 2012).

In recent years, several experimental evidences suggested that astrocytes, together with neurons, actively regulate synaptic transmission via neurotransmitter uptake/release through their processes wrapping around synapses (Araque et al., 1999; Parpura and Haydon, 2000; Ge and Duan, 2007; Navarrete and Araque, 2008; Allen and Barres, 2009). In particular, their effect on glutamate clearance may be relevant for the pathogenesis of MDD, because an excess of this neurotransmitter potentially exerts neurotoxic effects (Hynd et al., 2004; Mattson, 2007). Therefore, ADs may act both on astrocytes and neurons to re-establish a properly functional astrocyte–neuronal network, thereby restoring physiological glutamate homeostasis (Kugaya and Sanacora, 2005; Sanacora et al., 2012).

The principal aim of this study was to characterize *in vivo* the effects of systemic (intraperitoneal, i.p.) acute administration of FLX and desipramine (DMI) *per se* on the modulation of ERK1/2 in the neurons and astrocytes of the adult mouse PFC. This characterization should represent the groundwork to determine the effects of such drugs *per se* on specific cellular responses and to compare them with effects obtained when drugs are administered in animal models of MDD where a modulation of ERK signaling has been proven.

EXPERIMENTAL PROCEDURES

Animals

Eight- to ten-week-old, male CD-1 mice (35 ± 5 g) (Charles River, Germany) were acclimatized to the laboratory conditions [12-h light/dark cycle, 22 ± 0.5 °C room temperature (RT)], single-housed three days after delivery and were treated two weeks after arrival. Food and water were available *ad libitum*. Animal care was conducted in accordance with the national ethics committee on animal care and use (European Communities Council Directive 86/609/EEC). Protocols were approved by the committee for the ''Care and Use of Laboratory Animals'' of the Government of Upper Bavaria, Germany. All efforts were made to minimize animal suffering and the number of experimental animals was reduced to the minimum required for a reliable statistic analysis.

Drugs

FLX and DMI (#F132 and #D3900, respectively, Sigma–Aldrich, Germany) were dissolved in water to prepare stock solutions. For working solutions, drugs were diluted in saline.

Treatments

On the day of the experiment, mice received an i.p. injection of 10 mg/kg of each drug and were put back in their home cage until sacrificed 15 min later. Control mice were injected with similar amounts of saline. The same experimental conditions and drug doses were used for Western blot and immunofluorescent–immunohistochemistry (IF–IHC). For Western blot, 10–16 animals were analyzed for each experimental group; for IHC, each group had four to five animals. The dosage of ADs was based on behavioral studies demonstrating its efficiency in activating ERK 15 min after injection (Valjent et al., 2004).

Tissue preparation for Western blot

Mice were anesthetized with isoflurane (Abbott House, Berkshire, UK, Forene 100%) and brains were rapidly removed and put into ice-cold PBS. Each brain was then placed into a coronal adult mouse brain matrix (World Precision Instruments, Sarasota, USA) to homogeneously slice it. Afterward PFC, striatum and Hip were dissected under a binocular microscope (Stemi DRC, Carl Zeiss MicroImaging GmbH, Munich, Germany). Tissues were then stored at −80 °C until further processing. On the day of Western blot, samples were lysed in ice-cold lysis buffer (50 mM Hepes, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 1.5 mM $MgCl_2$, 2 mM phenylmethylsulfonylfluoride, 10 mM NaF) and centrifuged for 10 min (min) at 12,000 g at 4 °C to remove cellular debris.

Western blot procedure

The Western blot procedure was performed essentially as previously described (Di Benedetto et al. 2012) with the only difference that proteins were separated with 15% SDS–PAGE. Films were scanned and processed by densitometric analysis using ImageJ software (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/). Results for each sample were first normalized against their own HPRT value and then expressed as changes in % above their respective control values for each region.

Tissue preparation for IF–IHC

Mice were rapidly anesthetized with Narcoren (Merial GmbH, Hallbergmoos, Germany) and intracardially perfused with ice-cold 4% paraformaldehyde (PFA) in 0.1 M PBS (pH 7.5) with a peristaltic pump at 70 ml/min, after a brief rinse with ice-cold Ringer's solution. Afterward, brains were removed, post-fixed in the same fixative solution for 24 h at 4 °C, cryoprotected in 0.1 M PBS containing 25% sucrose for 3 days at 4 °C, frozen on dry ice and then stored at −80 °C until further processing. Four series of coronal slices (40 µm) were cut on a cryostat (Microm HM 500 OM, Thermo Fisher Scientific, Walldorf, Germany) and kept in a solution containing 25% ethylene glycol, 25% glycerol, and 0.1 M PBS at −20 °C until processed for IF–IHC.

IF–IHC procedure

The IF–IHC procedure was adapted from protocols previously described (Di Benedetto et al., 2009). In brief, the whole procedure was identical except for the addition of the mouse anti-glial fibrillary acidic protein (GFAP) (dilution 1:400, #G3893, Sigma–Aldrich) antibody together with the S100ß antibody in the first overnight antibody incubation step.

Quantification with confocal laser scanning microscopy (CLSM)

Mouse PFC was defined using the mouse brain atlas by Paxinos and Franklin (2001). Every fourth slice series was taken for counting neuronal pERK and a parallel series for astrocytic pERK, either in the right or in the left hemisphere, in randomized order.