Progress in Neuro-Psychopharmacology & Biological Psychiatry 71 (2016) 117–122

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Adverse childhood experiences associate to reduced glutamate levels in the hippocampus of patients affected by mood disorders

Sara Poletti [a,b,*], Clara Locatelli [a], Andrea Falini [b,c], Cristina Colombo [a], Francesco Benedetti [a,b]

[a] Department of Clinical Neurosciences, Scientific Institute Ospedale San Raffaele, Milano, Italy
[b] C.E.R.M.A.C. (Centro di Eccellenza Risonanza Magnetica ad Alto Campo), University Vita-Salute San Raffaele, Milano, Italy.
[c] Department of Neuroradiology, Scientific Institute Ospedale San Raffaele, Milano, Italy.

ARTICLE INFO

Article history:
Received 10 June 2016
Received in revised form 14 July 2016
Accepted 19 July 2016
Available online 20 July 2016

Keywords:
Mood disorders
Glutamate
Spectroscopy
Early stress

ABSTRACT

Adverse childhood experiences (ACE) can possibly permanently alter the stress response system, affect the glutamatergic system and influence hippocampal volume in mood disorders. The aim of the study is to investigate the association between glutamate levels in the hippocampus, measured through single proton magnetic resonance spectroscopy (1H-MRS), and ACE in patients affected by mood disorders and healthy controls. Higher levels of early stress associate to reduced levels of Glx/Cr in the hippocampus in depressed patients but not in healthy controls. Exposure to stress during early life could lead to a hypofunctionality of the glutamatergic system in the hippocampus of depressed patients. Abnormalities of glutamatergic signaling could then possibly underpin the structural and functional abnormalities observed in patients affected by mood disorders.

1. Introduction

Adverse childhood experiences (ACE), such as emotional or physical abuse and neglect, are a rather common occurrence in western countries (Scher et al., 2004). Exposure to harsh parenting practices or to maltreatment during childhood can increase individuals' vulnerability to mood disorders, possibly by altering the stress response system (Alloy et al., 2006) and sensitizing individuals to later stress. Exposure to ACE increases the likelihood of early onset, and lead to a more severe course of the disorder (Lu et al., 2008; Post et al., 2001), including more frequent self-injurious behavior (Newport et al., 2002), and suicide attempts (Brodsky et al., 2001; Gladstone et al., 1999; Swann et al., 2005).

The hippocampal formation is one of the structures more sensitive to stress, including childhood abuse (Andersen et al., 2008), because it is a particularly plastic and vulnerable brain region (McEwen, 2007). Diminished hippocampal volume was observed in adults with maltreatment histories (Bremner et al., 1997; Frodl et al., 2010; Weniger et al., 2009) and reduced hippocampal volume has been reported in a multitude of psychiatric disorders, including major depression (MDD) (Opel et al., 2014) and bipolar disorder (BD) (Geuze et al., 2005; Poletti et al., 2014). Stress related damages in the hippocampus have been suggested to largely depend on the glutamatergic system as suggested by altered levels of glutamate reported in the hippocampus of rats exposed to early maternal deprivation (Llorente et al., 2012, Zhang et al., 2013). Whereas acute stress seems to have the general effect of increasing glutamatergic neurotransmission, chronic stress exposure has been suggested to cause prolonged periods of stimulated glutamate release following acute stress exposure, at least in the hippocampus (Popoli et al., 2012).

Glutamate effects are mediated through a large variety of ionotropic (ion channels) and metabotropic (G protein-coupled) receptors including the most studied α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors. Abnormalities in the expression of glutamate receptors have been reported in areas of the brain associated with BD, including the dorsolateral prefrontal cortex, the hippocampus, and the striatum (Kristiansen and Meador-Woodruff, 2005, McCullumsmith et al., 2007). Changes in the surface expression of AMPAR and NMDAR subunits have been shown to follow chronic stress exposure, probably as a compensatory response to elevated synaptic glutamate activity. These changes seem to be associated with a decreased transmission efficiency and potentially impaired synaptic plasticity (Popoli et al., 2012). Considering the important role of glutamate in synaptic transmission and plasticity, perturbation of the glutamatergic system is considered one of the factors involved in synaptic abnormalities found in mood disorders (Sanacora et al., 2008). Indeed, in depressed patients, alterations of synaptogenesis and neural plasticity have been reported and abnormalities were observed in glutamate clearance at the synaptic space and in modulation of astrocytic energy metabolism involving glutamate (John et al., 2012; Walter et al., 2009). Also, a dysregulated expression of glutamate related genes was observed in the hippocampus of patients with depression (Duric et al., 2013). These

* Corresponding author at: Istituto Scientifico Universitario Ospedale San Raffaele, Dipartimento di Neuroscienze Cliniche, San Raffaele Turro, Via Stamira d'Ancona 20, 20127 Milano, Italy.
E-mail address: poletti.sara@hsr.it (S. Poletti).

http://dx.doi.org/10.1016/j.pnpbp.2016.07.007
0278-5846/

data then suggest that exposure to stress could partly exert its influence on the depressed brain by altering glutamatergic neurotransmission.

The in vivo levels of glutamate in the brain can be detected using single proton magnetic resonance spectroscopy (1H-MRS), a non-invasive brain imaging technique that can detect alterations in brain biochemistry in the presence of apparently normal anatomy. Resonances in the 1H-MR spectrum can be reliably quantified for several metabolites with brain concentrations in the millimolar range including the excitatory amino-acid glutamate (Glu); glutamine (Gln), the glial cell reservoir storage form of glutamate; the sum of Glu and Gln (Glx); and creatine + phosphocreatine (Cr), a measure of energy utilization which has historically been used as an 1 HMRS internal standard.

Several studies focused on brain concentration of Glu and Glx in mood disorders reporting contrasting results, which may be due to different mood states and medication histories, as well as regional brain differences. In a recent meta-analysis on studies performed on bipolar patients, Glx was found to be increases in frontal regions compared to healthy subjects but no differences were observed when searching for Glx/Cr ratio in whole brain (Gigante et al., 2012). Within the hippocampus either higher levels of Glx (Colla et al., 2009) and no differences (Silveira et al., 2016; Zanetti et al., 2015) were reported between BD patients and controls. Also no difference in Glu levels were reported between bipolar subjects and patients affected by MDD (Hermens et al., 2015). Finally, in MDD subjects Glx was found to be reduced in hippocampus (de Diego-Adelino et al., 2013), anterior cingulate cortex and prefrontal cortex (for a review see (Jun et al., 2014)).

Although the interaction between early stress and glutamate seems to be crucial for the neuroanatomy and biochemistry of the hippocampus in mood disorders, no study so far focused on the interaction between ACE and glutamate in the hippocampus of patients affected by mood disorder. In the present study, concentrations of Glx in the hippocampus of BD and MDD patients in a depressive state, and of healthy controls were investigated in vivo with 1H-MRS in order to specifically address the interrelationships between hippocampal glutamate concentrations and ACE. Following the literature we expect to observe an interaction between diagnosis and exposure to ACE in influencing Glx levels in the hippocampus.

2. Materials and methods

We studied 38 consecutively admitted inpatients affected by a major depressive episode (19 in course of BD type I, and 19 affected by MDD; DSM-IV - Diagnostic and Statistical Manual of Mental Disorders, 4th Edition - criteria; SCID - Structured Clinical Interview for DSM-IV Axis I Disorders (First, 1996)- interview), and 17 healthy controls (HC). Among MDD patients 22% were drug free, 33% were taking SSRI's, 22% SNRI's and 27% antipsychotics. Among BD patients 40% were drug free at the time of scanning, whereas 36% of patients were taking mood stabilizers. Other treatments include SSRIs (25%), SNRIs (16%), and antipsychotic (23%). To be included in the study the patients had to meet the following criteria: to be willing to participate; a baseline Hamilton Depression Rating Scale (HDRS) score of 18 or higher; absence of other diagnoses on Axis I; absence of mental retardation on Axis II; absence of pregnancy, history of epilepsy, major medical and neurological disorders; no treatment with long-acting neuroleptic drugs in the last three months before admission; absence of a history of drug or alcohol dependency or abuse within the last six months. Physical examinations, laboratory tests and electrocardiograms were performed at admission. After complete description of the study to the subjects, a written informed consent was obtained. All the research activities were approved by the local ethical committee.

2.1. ACE assessment

The exposure to early (between age 5 and 15) stressful life events was rated using the Risky Family Questionnaire (RFQ), an instrument already validated in patients with BD (Benedetti et al., 2014). The questionnaire aims to assess the degree of risk of physical, mental, and emotional distress that subjects faced in their homes during childhood and adolescence. It is composed of 10 + 3 filler items and subjects are asked to rate the frequency with which certain events or situations occurred in their homes, using a 5-Point Likert scale. Differently from other questionnaires rating mainly the number of stressful events such as abuse this instrument assesses the degree of harsh parenting with overt family conflict and deficient nurturing experienced by the children in their familial environment.

2.2. MRS procedure

MRS was performed at the C.E.R.M.A.C. (Centro d'Eccellenza di Risonanza Magnetica ad Alto Campo) in San Raffaele hospital in Milan on a 3.0 Tesla magnet (Intera Philips) with a standard quadrature head coil.

A structural MRI study was performed first to rule out brain lesions and to localize the volume of interest (VOI) for the spectroscopy study, acquiring sagittal T1 images, axial T2 fast spin-echo (FSE) images parallel to the bicomessural line, and coronal fluid-attenuated inversion recovery (FLAIR) images orthogonal to the axial ones.

1H-MRS data were acquired using a point resolved spectroscopy (PRESS) sequence (TR 2000 ms, TE 30 ms, 128 acquisitions) from VOI of 30 × 20 × 15 mm size positioned at the level of the hippocampus. The rectangular shape of the VOI, having the major axis along the anterior–posterior direction and a relatively short axis along the lateral direction, was chosen in order to include mostly gray matter minimizing white matter contamination. For the selected metabolites mean value of the area of acquisition was collected.

H1-MR spectra were then analyzed using LCModel version 6.3.0. The LCModel method analyzes an in vivo spectrum as a Linear Combination of Model in vitro spectra from individual metabolite solutions. Complete model spectra, rather than individual resonances, are used in order to incorporate maximum prior information into the analysis. A nearly model-free constrained regularization method automatically accounts for the baseline and lineshape in vivo without imposing a restrictive parameterized form on them. LCModel is automatic (non-interactive) with no subjective input. Approximately maximum-likelihood estimates of the metabolite concentrations and their uncertainties (Cramér-Rao lower bounds) are obtained.

The unsuppressed water signal measured from the same VOI was used as an internal reference for the quantification (assuming 80% brain water content). The LCModel analysis calculates the best fit to the experimental spectrum as a linear combination of model spectra (solution spectra of brain metabolites). The final analysis is performed in the frequency domain; however, raw data (FIDs) are used as standard data input. An example spectrum is presented in Fig. 1.

2.3. Statistical analyses

All statistical analyses were performed both, by grouping together BD and MDD patients and comparing depressed patients and HC, and by dividing participants in three groups according to diagnosis.

A simple correlation analysis was performed between ACE and Glx/Cr in the different groups. Then, given the a priori hypothesis that categorical (diagnosis) and continuous (extent of exposure to early stress) predictors would interact in affecting the outcome of interest (Glx/Cr), the influences of the predictors were modeled using a separate-slopes design within the context of the Generalized Linear Model (GLZM), instead of the traditional Analysis of Covariance, which is inappropriate when the assumption of homogeneity of variances is not respected and when a linear relationship between dependent and independent variables could not be a priori assumed. Therefore, in order to test their effect on Glx/Cr ratio, diagnosis and ACE were entered as factors into a GLZM separate-slopes analysis with a log link function, also