European Neuropsychopharmacology (2011) 21, 545–562

www.elsevier.com/locate/euroneuro

Nortriptyline influences protein pathways involved in carbohydrate metabolism and actin-related processes in a rat gene–environment model of depression

Chiara Piubelli [a], Susanne Gruber [b], Aram El Khoury [b], Aleksander A. Mathé [b], Enrico Domenici [a], Lucia Carboni [a,*]

[a] *Neurosciences CEDD, GlaxoSmithKline, Verona, Italy*
[b] *Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden*

Received 20 July 2010; received in revised form 24 September 2010; accepted 9 November 2010

KEYWORDS
2-D electrophoresis;
Hippocampus;
Maternal separation;
Nortriptyline;
Proteomics;
Pre-frontal cortex

Abstract

Although most available antidepressants increase monoaminergic neurotransmission, their therapeutic efficacy is likely mediated by longer-term molecular adaptations. To investigate the molecular changes induced by chronic antidepressant treatment we analysed proteomic changes in rat pre-frontal/frontal cortex and hippocampus after nortriptyline (NT) administration. A wide-scale analysis of protein expression was performed on the Flinders Sensitive Line (FSL), a genetically-selected rat model of depression, and the control Flinders Resistant Line (FRL). The effect of NT treatment was examined in a gene–environment interaction model, applying maternal separation (MS) to both strains.

In the forced swim test, FSL rats were significantly more immobile than FRL animals, whereas NT treatment reduced immobility time. MS alone did not modify immobility time, but it impaired the response to NT in the FSL strain.

In the proteomic analysis, in FSL rats NT treatment chiefly modulated cytoskeleton proteins and carbohydrate metabolism. In the FRL strain, changes influenced protein polymerization and intracellular transport. After MS, NT treatment mainly affected proteins in nucleotide metabolism in FSL rats and synaptic transmission and neurite morphogenesis pathways in FRL rats. When the effects of NT treatment and MS were compared between strains, carbohydrate metabolic pathways were predominantly modulated.

1. Introduction

Although most available antidepressants acutely increase monoaminergic neurotransmitter concentration in synapses,

* Corresponding author. Neurosciences CEDD, GlaxoSmithKline, via A. Fleming 4, 37135 Verona, Italy. Tel.: +39 0458218294; fax: +39 0458218047.
E-mail address: lucia.e.carboni@alice.it (L. Carboni).

0924-977X/$ - see front matter © 2010 Elsevier B.V. and ECNP. All rights reserved.
doi:10.1016/j.euroneuro.2010.11.003

their therapeutic efficacy is supposed to be mediated by longer-term molecular adaptations (Berton and Nestler, 2006; Schloss and Henn, 2004). The delay usually observed between start of treatment and clinical improvement supports the hypothesis that the regulation of neurotransmitter availability in brain synapses is followed by a cascade of molecular events which stand at the basis of symptom amelioration. Since the discovery of effective antidepressants, several lines of research were committed to the investigation of long-term effects of antidepressant treatments, with the aim of gaining further knowledge about the molecular basis of their therapeutic efficacy. Antidepressant treatments are reported to induce receptor desensitisation, regulation of G protein and second messengers, modulation of cytoskeletal microtubule dynamics, alterations in gene expression, changes in synaptic plasticity, increased neurotrophic regulation, support of neurogenetic events, modulation of stress responses, and anti-inflammatory actions; these mechanisms are believed to play a role in their therapeutic efficacy (Bianchi et al., 2005; Castren, 2004; Donati and Rasenick, 2003; Dranovsky and Hen, 2006; Malberg and Blendy, 2005; O'Brien et al., 2004; Pittenger and Duman, 2008; Schloss and Henn, 2004; Tanis and Duman, 2007). Recently, the availability of large-scale analysis methods allowed the investigation of antidepressant mechanism of action with unbiased approaches (Carboni et al., 2006b; Conti et al., 2007; Khawaja et al., 2004; Landgrebe et al., 2002; Sillaber et al., 2008). A major challenge facing these lines of research is the choice of a suitable experimental design to discriminate molecular changes related to antidepressant therapeutic efficacy from neutral or even toxicology-related modifications. Selecting an appropriate model is critical since the therapeutic potential of antidepressant treatment is not elicited in healthy people; thus studies in healthy animals may pose difficult challenges to distinguish relevant from non relevant results. Since all complex features of major depressive disorder (MDD) cannot be reproduced in rodents, the choice falls on disease models able to suitably mimic some relevant symptoms and/or reproduce significant etiological factors of the disease (Cryan and Slattery, 2007; Holmes, 2003). MDD is recognised to be associated to an interaction between genetic predisposition and environmental challenges (aan het Rot et al., 2009; Bartolomucci and Leopardi, 2009). Therefore, we addressed our investigations on molecular changes brought about by antidepressant treatment in an animal model including features related to genetic predisposition and environmental challenge. These studies were performed within GENDEP, an integrated project combining large-scale pharmacogenomic studies on depressed patients with preclinical investigations on animal models of disease, focusing on treatment with pro-serotonergic and pro-noradrenergic antidepressants (Uher et al., 2010). In this framework, to gain a better understanding of the molecular changes induced by antidepressant treatment, we have analysed proteomic changes in rat brain regions after chronic administration with a pro-noradrenergic tricyclic antidepressant, nortriptyline (NT) (Gillman, 2007). Wide-scale analyses of protein expression were performed by 2-D electrophoresis on the Flinders Sensitive Line (FSL), a genetically selected rat model of depression that displays good face, predictive and construct validity (Overstreet et al., 2005; Yadid et al., 2000). The Flinders Resistant Line (FRL), which does not show the depressive-like behaviour, was used as a reference, since this strain was derived in parallel with FSL from the same Sprague–Dawley strain (Overstreet et al., 2005). In MDD, the environmental challenges are often related to stressful experiences, especially in early ages (Hammen, 2005; Horesh et al., 2008); we thus included in the experimental design the exposure to maternal separation (MS).

2. Experimental procedures

2.1. Experimental design

FSL (total n = 36) and FRL (total n = 36) rats were maternally separated from post-natal day (PND) 2 to 14 (n = 19; n = 18 respectively). Control groups were not separated. Both stressed and control rats were split into groups receiving NT or vehicle for 31 days (n = 8–10/group; Fig. 1). One week before the end of treatment, a forced swim test was administered to all rats. At the end of treatment, rats were euthanised and protein extracts were prepared from hippocampus (HIP) and pre-frontal/frontal cortex (P/FC) for proteomic analysis. Image and statistical analyses were carried out on samples run together. Maps belonging to the following groups were compared in both brain regions:

FSL + NT vs. FSL + vehicle; FRL + NT vs. FRL + vehicle;
FSL + MS + NT vs. FSL + MS + vehicle; FRL + MS + NT vs. FRL + MS + vehicle;
FSL + MS + NT vs. FRL + MS + NT.

2.2. Animals

FSL and FRL rats from colonies maintained in the animal facility at the Karolinska Institutet were used. Rats were housed in standard cages at constant room temperature (22 ± 1 °C) and relative humidity (45–55%) under a regular 12 h light/dark schedule (lights on at 07:00). Rat chow and tap water were freely available. The Stockholm's Ethical Committee for Protection of Animals approved the study and all procedures were conducted in conformity with the Karolinska Institutet's Guidelines for the Care and Use of Laboratory animals, which follow the European Communities Council Directive of 24 November 1986. Efforts were made to minimise the number of animals used and reduce their suffering.

2.3. Maternal separation and pharmacological treatment

MS procedure was performed as previously reported in El Khoury et al. (2006). Pups assigned to the MS group were separated from the dam as a litter for 180 min, beginning at 09:00 a.m., from PND 2 to PND 14. Control rats were left undisturbed until weaning, except for the routine cleaning of the cages twice weekly. Body weights were recorded on PND 2 and PND 14 and all pups were weaned on PND 23. Siblings were then separated by sex and housed in groups of 3 to 5 rats per cage. Only males were included in the study. At PND 44–45 rats were split into groups receiving NT admixed to food pellets (0.34 g/kg chow for the first three weeks, 0.41 g/kg chow during the rest of the experiment) or vehicle for 31 days. An antidepressant dose of approximately 25 mg/kg body weight/day was estimated by calculating the average food intake (~22 g/day) by dividing the measured total mean daily consumption of pellets per cage by the number of animals per cage, in agreement with previous results (Petersen et al., 2009).