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Research report

Effect of amitriptyline treatment on neurofilament-H protein in an experimental model of depression

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ABSTRACT

It has been proposed that depression is associated with dysfunction of hippocampal plasticity. Novel hypotheses suggest that antidepressants induce neuronal structural plasticity, although the underlying mechanisms still remain unclear. Therefore, the aim of this study was to investigate the effects of amitriptyline on levels of phosphorylated heavy neurofilament subunit (NF-H) in the hippocampus of mice exposed to acute and chronic behavioral despair paradigms. Immunoblotting experiments showed that animals exposed to the tail suspension test (TST) displayed diminished levels of pNF-H 24 h after testing. Repeated administration of amitriptyline (10 mg/kg i.p.) prevented this decreased hippocampal phosphorylation of NF-H. Conversely, administration of citalopram (10 mg/kg i.p.) left unchanged pNF-H levels. The expression of pNF-H was also analyzed by immunofluorescence in mice exposed to the unpredictable chronic mild stress paradigm (UCMS), an experimental model of depression. Mice that developed a depressive-like behavior showed a decreased pNF-H immunostaining selectively in the hippocampal CA3 region. Chronic administration of amitriptyline reversed the despaired behavior induced by exposure to UCMS paradigm and, fully recovered pNF-H labeling to control values. Our results indicate that the cytoskeletal remodeling induced by amitriptyline in the hippocampal CA3 region might underpin its behavioral efficacy. Hippocampal alterations of the NF appeared associated with the mechanism of this antidepressant drug and may contribute to its psychotherapeutic actions.

1. Introduction

Neuronal plasticity or remodeling is a critical process that underlies the normal central nervous system function by which the brain acquires information and makes the appropriate adaptive responses in future-related settings. During the last few years it has indeed become apparent that several psychiatric conditions, such as mood disorders, are associated with deficits or impairment of neuronal plasticity (Castren, 2005; Krishnan and Nestler, 2008). Although for many years depression has been linked to abnormalities in monoaminergic neurotransmission, it is now well accepted that this condition is characterized by profound alterations of brain function and responsiveness. Hence depressed subjects display an inability to cope or adapt to the environment and may be more vulnerable to challenging experiences (Pittenger and Duman, 2008). These abnormalities may be intimately linked with neuronal plasticity and the ability to modulate a cascade of events from intracellular signaling mechanisms to gene expression. Indeed, the brains of depressed subjects show structural abnormalities such as reduction in hippocampal volume, low levels of brain-derived neurotrophic factor, abnormal function of the hypothalamic–pituitary–adrenal axis and, glutamate mediated toxicity (Aan het Rot et al., 2009; Sheline et al., 2003). Dysfunctions of neuronal plasticity might, therefore, imply in the pathophysiology of mood disorders. Dynamic processes such as adult neurogenesis, the development of dendritic spines, synaptic and cytoskeletal adaptations are included under the umbrella of neuronal plasticity and are essential to normal functioning. Several lines of evidence during the last decade have suggested that antidepressants may act by promoting this plasticity (Duman et al., 2000; Castren, 2005). Despite the well-established antidepressant action on synthesis of neurotrophic factors (Nibuya et al., 1996; Chen et al., 2001) and to promote neurogenesis in the hippocampus (Malberg et al., 2000), the effect of antidepressant treatment on cytoskeletal plasticity is not well characterized. Considering

Abbreviations: NF-H, phosphorylated heavy neurofilament subunit; TST, tail suspension test; SPT, sucrose preference test; UCMS, unpredictable chronic mild stress.

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the role of cytoskeleton in neuronal cytoarchitecture and function (Lazarides, 1980; Nixon and Sihag, 1991; Julien, 1999), it is reasonable to predict that cytoskeletal components might be affected in depressive states, leading to hippocampal structural alteration and functional impairment. Neuronal cytoskeleton is composed of three types of filaments: actin microfilaments, microtubules and neurofilaments. Neurofilaments (NFs) that comprise three subunits: light (NF-L), medium (NF-M) and heavy (NF-H) (Alberts, 1994), have been proposed to participate in plastic structural changes (Kong et al., 1998). It has been showed that NF-L is phosphorylated by CaMKII in a subpopulation of apical dendrites during long-term potentiation (LTP) and long-term depression (LTD) (Hashimoto et al., 2000a, 2000b). Hippocampal depression-associated plastic alterations may be due to neurofilament changes, since a significant NF-L immunoreactivity decrease was observed in the hippocampus of animal exposure to a learned helplessness paradigm, that might be related to the diminution of dendrite shaping (Reinés et al., 2004). As plasticity-responsive elements, cytoskeletal proteins have been shown to differentially respond to antidepressant drugs. Magarinos et al. (1999) have reported that while fluoxetine failed to reverse the rat hippocampal dendritic atrophy as measured by Golgi staining, the atypical antidepressant tianeptine prevented the hippocampal volume loss induced by stress and, reversed stress-induced rat hippocampal atrophy. Despite the evidence indicates that antidepressants might modulate some components of cytoskeletal plasticity, it seemed interesting clarify if cytoskeletal alterations is a common action among antidepressants. Neurofilament function depends on the state of phosphorylation of the numerous serine/threonine residues in these proteins. About 80% of NF are highly phosphorylated and integrated by cross-linking to stabilize the cytoskeleton (Petzold et al., 2003). The neurofilament heavy chain (NF-H) is the most extensively phosphorylated protein of the human brain, and possibly the entire human body (Petzold, 2005). Thus, the aim of this work was to examine the effect of treatment with the antidepressant amitriptyline on the phosphorylation levels of NF-H, a neuronal cytoskeletal protein used as marker to study neuronal plasticity in the hippocampus, in mice exposed to the tail suspension test (TST) and, to the unpredictable chronic mild stress (UCMS), animal models which simulates the behavioral despair paradigm of depression.

2. Experimental procedures

2.1. Animals

Male CD1 mice (20–22 g, 3 weeks of age) from the Harlan Laboratories (Bresso, Italy) breeding farm were used. Mice were randomly assigned to standard cages, with four to five animals per cage. The cages were placed in the experimental room 24 h before behavioral test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1 °C with a 12 h light/dark cycle, light on at 7 a.m. The experimental protocol was carried out after approval by the Animal Care and Research Ethics Committee of the University of Florence, Italy, under license from the Italian Department of Health and in compliance with international laws and policies (Directive 2010/63/EU) of the European parliament and of the council of 22 September 2010 on the protection of animals used for scientific purposes. All studies involving animals are reported in accordance with the ARRIVE guidelines for experiments involving animals (McGrath and Lilley, 2015).

2.2. Behavioral testing

Animals were habituated to the experimental room and randomly assigned to each treatment group. Mice were investigated by observers blinded for treatment of the animals. Behavioral tests were performed after 4 weeks of UCMS exposition in this order: hole-board test, tail suspension test, sucrose preference test. Behavioral results are given as mean ± S.E.M. One-way analysis of variance followed by Tukey post hoc test, were used for statistical analysis. The number of animals per experiment was based on a power analysis (Charan and Kantharia, 2013) and 10 animals per group were used. A total of 10 animals was used to have the probability of 86% that the study detects a treatment difference at a two-sided 0.05 significance level. Sample size was calculated by G Power software.

2.2.1. Tail suspension test (TST)

A piece of tape was adhered to the upper middle of the tail of each animal, creating a flap with the overlap of tape. Mice were suspended from a plastic rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape. The duration of the test was 6 min and immobility was also measured the first 2 min and the last 4 min. Immobility was defined as the absence of any limb or body movements, except those caused by respiration (Steru et al., 1985).

2.2.2. Sucrose preference test (SPT)

The sucrose preference test (Willner et al., 1987) was employed herein to determine anhedonia, one of the core symptoms of major depression in human. At the beginning of the test all the groups were singly housed during 48 h in individual cages. After 24 h of food and water deprivation, mice were given a choice between two pre-weighed bottles for 24 h, one with 1% sucrose solution and another with normal drinking water. The consumption of water and sucrose was measured by weighing the bottles. Sucrose preference is calculated as a percentage of the volume of sucrose intake over the total volume of fluid intake.

2.2.3. Locomotor activity

The locomotor activity was evaluated by using the hole-board test. The apparatus consisted of a 40 cm square plane with 16 flush mounted cylindrical holes (3 cm diameter) distributed 4 by 4 in an equidistant, grid-like manner. Mice were placed on the center of the board one by one and allowed to move about freely for a period of 5 min each. Two photo beams, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signaled the movement of the animal (counts in 5 min) on the surface of the plane (spontaneous mobility). Miniature photoelectric cells, in each of the 16 holes, recorded (counts in 5 min) the exploration of the holes (exploratory activity) by the mice (Doukkali et al., 2015).

2.3. Unpredictable chronic mild stress (UCMS)

UCMS used in this study was designed as described previously (Nollet et al., 2011). In brief, the mice were subjected daily to various CMS procedures according to an unpredictable schedule for 4 weeks. The CMS protocol consists of the sequential application of a variety of mild stressors including restraint, forced swimming, water and/or food deprivation, and pairing with another stressed animal in wet sawdust, housing in wet sawdust, reversal of the light/dark cycle, and housing in constant illumination or darkness each for a period ranging from 10 min to 24 h in a schedule that lasts for 4 weeks. UCMS-induced modifications in mice were assessed using immobility time in the tail suspension test (TST) and, anhedonia in the SPT.