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Molecular changes associated with escitalopram response in a stress-based model of depression

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ABSTRACT

Converging evidence points at hypothalamus-pituitary-adrenal (HPA) axis hyperactivity and neuroinflammation as important factors involved in the etiopathogenesis of major depressive disorder (MDD) and in therapeutic efficacy of antidepressants. In this study, we examined the molecular effects associated with a response to a week-long treatment with escitalopram in the chronic escape deficit (CED) model, a validated model of depression based on the induction of an escape deficit after exposure of rats to an unavoidable stress. We confirmed our previous result that a treatment with escitalopram (10 mg/kg) was effective after 7 days in reverting the stress-induced escape deficit in approximately 50% of the animals, separating responders from non-responders. Expression of markers of HPA axis functionality as well as several inflammatory mediators were evaluated in the hypothalamus, a key structure integrating signals from the neuro, immune, endocrine systems. In the hypothalamus of responder animals we observed a decrease in the expression of CRH and its receptors and an increase in GR protein in total and nuclear extracts; this effect was accompanied by a significant decrease in circulating corticosterone in the same cohort. Hypothalamic IL-1β and TNFα expression were increased in stressed animals, while CXCL2, IL-6, and ADAM17 mRNA levels were decreased in escitalopram treated rats regardless of the treatment response. These data suggest that efficacy of a one week treatment with escitalopram may be partially mediated by a decrease HPA axis activity, while in the hypothalamus the drug-induced effects on the expression of immune modulators did not correlate with the behavioural outcome.

1. Introduction

Understanding the neurobiological basis of major depressive disorder (MDD) and the mechanisms behind the efficacy of antidepressant strategies is a pressing need for the scientific community worldwide.

One of the most reliable reported neurobiological alterations in MDD is impaired hypothalamus-pituitary-adrenal (HPA) axis functionality: HPA axis hyperactivity, glucocorticoid (GC) insensitivity and CRH overexpression in the hypothalamus or the cerebrospinal fluid have been reported in depressed patients (Sanders and Nemeroff, 2016), and similar effects were observed in preclinical studies in animal models of depression (Wang et al., 2008). Chronically elevated glucocorticoids may exert detrimental effects on the central nervous system (CNS) functionality, cause atrophy and disruption of connectivity, especially in the hippocampus and prefrontal cortex, and can also increase the number of inflammatory cells and the production of pro-inflammatory cytokines both centrally and in the periphery (Himmerich et al., 2013). Elevated levels of inflammatory markers have been reported in peripheral blood and spinal fluid of depressed patients (Köhler et al., 2017), while a variety of pro- and anti-inflammatory cytokines were altered in the frontal cortex of subjects with MDD (Shelton et al., 2011). HPA axis hyperactivity may be associated as well with neuroinflammatory process: cytokines, in fact, can activate the HPA axis and can impair glucocorticoid receptor (GR) functioning at multiple levels (Zunszain et al., 2011): by inhibiting GR translocation to the nucleus, GR-mediated gene transcription or by stimulating GRβ, an inactive form of GR (Anacker et al., 2011).

Accumulating evidence, clinical and preclinical, has been reported that the efficacy of antidepressant treatments may rely on normalization of hypothalamic function, by restoring GR mediated feedback inhibition of HPA axis activity (Anacker et al., 2011; Funato et al., 2006), and of cytokine production and plasma levels (Kenis and Maes,

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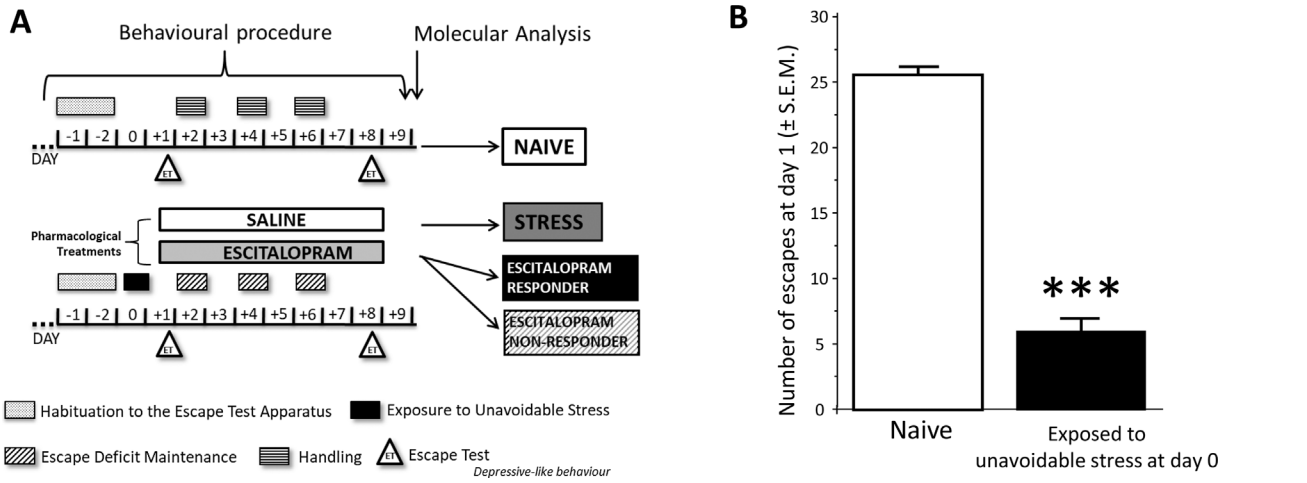

Fig 1. Flowchart of the experimental procedure (A). Induction of the acute escape deficit (B): the unavoidable stress exposed group (n = 85) underwent exposure to an acute unavoidable stress procedure and was tested for escape deficit 24 h later together with the naive group (n = 15) that was not exposed to the unavoidable stress procedure; scores are expressed as the mean number of escapes ± S.E.M. in a test session consisting of 30 consecutive electric shocks, every 30 s. Comparisons were made by one way analysis of variance (ANOVA), *** $p < 0.0001$ vs naive group (See materials and methods for a detailed description of the behavioural procedure).

2002).

However, the efficacy of currently available antidepressants is still limited by a significant delay between start of treatment and onset of beneficial effects and by high rates of treatment resistance (Willner and Belzung, 2015). Chronic behavioural models possess a great potential to help elucidate and overcome these important limitations of antidepressant treatment because on one side they more closely mimic the delayed pharmacological response observed in patients (O'Leary and Cryan, 2013), and in some of them, like the chronic unpredictable stress and chronic social defeat, is possible to separate rodents into bimodal subpopulations that respond or not to traditional antidepressant treatments (Willner and Belzung, 2015).

Along with these paradigms, also the chronic escape model of depression (CED), a valid and straightforward model, that is based on the induction of an escape deficit after exposure of rats to unavoidable stress (Benatti et al., 2012; Gambarana et al., 2001), can be used to study pharmacological antidepressant responsiveness. In fact, we previously demonstrated that: 1. Combination of acetylsalicylic acid with fluoxetine (FLX) accelerates and potentiates the effect of the antidepressant alone (Brunello et al., 2006); 2. After 7 days of treatment, escitalopram (ESC) (10 mg/kg) is already effective in restoring the natural tendency to avoid a noxious stimulus in about 50% of stressed rats developing an escape deficit (Benatti et al., 2014); 3. Co-administration of aspirin with ESC increases the treatment response rate to escitalopram at about 75% (Brunello et al., 2007).

To investigate the molecular mechanism behind the therapeutic efficacy of antidepressants, we examined the different molecular effects associated with a response to a week-long treatment with escitalopram in the CED model of depression on two key elements known to be altered in MDD: HPA axis functionality and cytokine production within the CNS. We focused on the hypothalamus, the neuro-endocrine interface in the brain and a key station for central circuits to orchestrate the maintenance of body homeostasis or allostasis, that is highly responsive to immune signals as well (MDAlboni et al., 2017a,b).

For this purpose, animals developing an escape deficit were treated for a week with escitalopram, tested for their ability to avoid a noxious stimulus and divided in responder and non-responder as previously reported (Benatti et al., 2014). Then, we evaluated the effects of escitalopram on expression of CRH, its receptors (CRHR1 and CRHR2), and heat shock protein 70 (HSP70), and also on glucocorticoid receptor (GR) mRNA and protein levels in the hypothalamus and on circulating corticosterone. We also measured in our model changes in hypothalamic expression of pro-inflammatory cytokines: Interleukin (IL-) 1β, Tumour Necrosis Factor (TNF) α, Interferon (IFN) γ, IL-6 (and its system [IL-6R and gp130, SOCS3, ADAM10 and ADAM17]), IL-18, rat homologues of IL-8 (CXCL1 and CXCL2), and two anti-inflammatory cytokines (IL-10, IL-4).

2. Methods

2.1. Animals

Experiments were performed on male Sprague-Dawley albino rats (Charles River Laboratories, Calco, Italy), weighing 150–175 g at their arrival. Animals were housed in polycarbonate cages (38 × 15 × 22 cm; 2 per cage) with ad libitum access to food and tap water throughout the study, and maintained under a 12 h inverted light-dark cycle (lights on at 6.00 p.m.) at the ambient temperature of 21 + 3 °C and relative controlled humidity. Animals were left undisturbed for 3 weeks before beginning any behavioural procedure. Experiments were carried out under a red light. Animals were handled and weighed daily, from the day before the beginning of the behavioural procedure throughout the whole experiment. The procedures used in this study were in strict accordance with European legislation on the use and care of laboratory animals (EU directive 2010/63/EU), with the guidelines of the National Institutes of Health on the use and care of laboratory animals (NIH Publications No.8023), and had the approval of the Ministry of Health and of the local Ethical Committee. All efforts were made to minimize animal suffering and to reduce the number of animals used in this study.

2.2. Behavioural procedures and pharmacological treatments

Animals were exposed to an unavoidable stress (US) session for the induction of an escape deficit. The US session consists of 50 min of immobilization in flexible wire nets and exposure to 80 electric shocks (1,5 mA x 7 s, one every 30 s), through an electrode applied to the distal third of the tail and connected to an S48 Grass stimulator as already described (Benatti et al., 2012).

Twenty-four hours later (Day 1), rats exposed to the US and a group of animals not exposed to the US (Naive), were tested for their reactivity towards an avoidable noxious shock in an escape-test apparatus, divided by a sliding door into two chambers one of which was connected to an electrode applied to the tail of the rat through a stimulator. All tested animals were allowed to explore the apparatus for at least 20 min/day in the 3 days preceding the test (Fig. 1A). The test