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Research report

Behavioural and transcriptional effects of escitalopram in the chronic escape deficit model of depression

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HIGHLIGHTS

- Escitalopram partially reverts the stress-sustained escape deficit condition.
- Escitalopram is effective in 50% of the animals after 7 days of treatment.
- An escitalopram treatment exerts anxiolytic effects in the CED model of depression.
- Escitalopram does not affect exploratory related behaviours in stressed animals.

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ABSTRACT

The study of depression is facing major challenges: first, the need to develop new drugs with a faster onset of action and second, fulfilling the unmet needs of treatment resistant patients with more effective compounds.

The chronic escape deficit (CED) is a valid and useful model of depression and is based on the induction of an escape deficit after exposure of rats to an unavoidable stress. This behavioural model provides a method for evaluating the capacity of a treatment to revert the escape deficit. The majority of antidepressant drugs need to be administered for at least 3–4 weeks in order to revert the escape deficit.

A 7-day treatment with escitalopram reverted the stress-induced escape deficit in approximately 50% of the animals. Escitalopram treatment decreased anxiety-related behaviours in stressed animals, by increasing the time spent in the central part of the arena with respect to saline treated stressed animals, without affecting exploratory related behaviours. Gene expression profiling was carried out in the hippocampus to identify new targets associated with the effects of stress or with the different response to escitalopram.

By combining a well-validated animal model with gene expression analysis we demonstrated that the CED model may represent a perfect tool for studying treatment-resistant depression.

1. Introduction

Major depression (MD) is one of the most prevalent and disabling of all medical disorders and is estimated to rank second among the major causes of disability worldwide by 2030 [1]. Despite its increasing incidence the currently available treatments have major clinical drawbacks: (i) latency of therapeutic action while undesirable side-effects appear earlier; (ii) only 60–65% of depressed patients benefit from the first antidepressant drug (AD) administered, while the remaining 35–40% fail to achieve a sufficient remission [2,3]. Moreover, the development of faster and more effective compounds is a slow and often unsuccessful process [4]. Despite the fact that the full spectrum of the depressive symptomatology cannot be replicated in rodents [5], valid and straightforward animal models of depression are still the best tool to gain insight in the pathophysiology of this disease and to lead to the development of the next generation of ADs [6,7].

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The chronic escape deficit (CED) model of depression possesses face, construct and pharmacological validity and is based on the induction, and maintenance, of an escape deficit upon exposing rats to an unavoidable stress [8–10]. In this model, a modification of the learned helplessness paradigm, rats exposed to unavoidable and unpredictable stresses, show hyporeactivity to avoidable aversive stimuli (i.e. electric shocks) and other behavioural, physiological, neurochemical, and hormonal changes that mimic some of the depressive symptoms observed in patients, including: hyporeactivity to pleasurable stimuli (anhedonia-like condition) [8]; modification in central monoamine levels (dopamine and serotonin) [11]; and, increased hypothalamus–pituitary–adrenal (HPA) activity [9]. Moreover, the observed changes are improved by chronic or sub-chronic but not acute administration of clinically relevant antidepressants, like selective serotonin reuptake inhibitors (SSRI) or tricyclic antidepressants [8].

In this context, the aim of our research was to use the CED model of depression to investigate both a panel of depression-related behavioural end-points as well as the features of genes associated with stress exposure or the response to a treatment with escitalopram in the hippocampus.

Escitalopram (ESC), the S(+) enantiomer of citalopram, is one of the most effective selective serotonin reuptake inhibitor (SSRI) for the treatment of MD with a short latency of therapeutic effect [12] and is approved also for the treatment of anxiety disorders [13]. In preclinical studies, a 4-week treatment with ESC is able to normalize sucrose intake in the chronic mild stress model of depression [14–18]. Interestingly, the reversion of anhedonia is present only in about 50% of treated animals, thus leading to a subdivision of animals according to their behaviour into responders and non-responders [14].

In the last few years, several studies were carried out to evaluate the molecular effects of AD treatments on gene expression in the brain [21–23]. In particular, the hippocampal formation, given its key role in mediating the effects of stress and antidepressant treatment, has been extensively studied [19,20]. Furthermore effects of AD in different brain areas appear to be state dependent and differ among unchallenged animals or in presence of a depressed-like phenotype [24,25].

In short, we combined behavioural characterization (assessing reactivity to noxious stimuli, locomotor activity, anxiety related behaviours and weight gain) with the latest microarray technology, which allowed us to investigate a panel of ESC-behavioural and transcriptional induced effects and simultaneously the features of genes associated with stress exposure or the response to antidepressant treatment in the hippocampus of CED rats.

2. Methods

2.1. Subjects

Experiments were performed on male Sprague-Dawley albino rats (Charles River, 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 the behavioural procedure (Day −2) 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 (EEC n. 86/609), with the guidelines of the National Institutes of Health on the use and care of laboratory animals, 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

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 × 7 s, one every 30 s), through an electrode applied to the distal third of the tail and connected to an S48 Grass stimulator.

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 trough a stimulator. All tested animals were allowed to explore the apparatus for at least 20 min/day in the 3 days preceding the test. The test session consists of 30 consecutive electric shocks (1.2 mA × 3.5 s), every 30 s, starting after a 5 min habituation period [8,26] (see Fig. 1 for a detailed time course of experimental procedures). Behaviour was labelled an escape when an animal moved to the neutral chamber of the apparatus within this 3.5 s period.

Naive rats scored between 20 and 30 escapes out of 30 trials [mean of the naive group at day 1 ± S.E.M. = 25.47 ± 0.44; $n = 36$]. Tested animals experiencing the unavoidable stress, showed a significantly lower mean of escapes with respect to the naive group [5.71 ± 0.58; $n = 172$; ANOVA univariate; $F(1, 205) = 239.03$; $p = 0$; Supplementary Fig. 1].

In our experimental conditions, approximately 75% of rats exposed to the US developed an escape deficit. Animals scoring 0–9 escapes (out of 30 trials) were randomly divided in two groups not statistically different from each other when the pharmacological treatments began (day 1, see Section 2.3). In order to maintain the induced despair like behaviour, US-exposed animals underwent a stress maintenance procedure, concomitantly with the pharmacological treatments, that consisted of 10 min of restraint stress in flexible wire nets plus 4 unavoidable shocks or 20 min of restraint stress in flexible wire nets alternated every 48 h after the first escape test (Fig. 1). By repeating this procedure on alternate days, the escape deficit can be maintained in all rats throughout any pharmacological manipulation [8,26]. All stress procedures and the escape tests were conducted during the dark phase.

2.3. Pharmacological treatments

Escitalopram (oxalate) (kindly provided by H. Lundbeck A/S. Copenhagen-Valby, Denmark) was dissolved in saline (12.8 mg/mL) and injected via an intraperitoneal route (i.p.) in a volume of 1 mL/kg body weight as previously described [19].

US-exposed rats scoring 0–9 escapes out of 30 trials were divided into 2 groups receiving for one week daily i.p. injections of saline ($n = 35$; stress) (1 mL/kg) or escitalopram (10 mg/kg/day) ($n = 38$; ESC). All treatments were performed before the beginning of the light phase (5 30 p.m. approximately). Each animal was tested 8 days after the induction of the escape deficit (Fig. 1). Naive rats ($n = 36$) did not receive any treatment, were handled on alternate days and were tested together with their stress-exposed counterparts (Fig. 1).

Escitalopram-treated animals were divided in two subgroups according to their performance in the escape test following a 7 day treatment. Based on the number of escapes totalized at test session