European Journal of Pharmacology 721 (2013) 158–167

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Chronic antidepressant treatments resulted in altered expression of genes involved in inflammation in the rat hypothalamus

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ARTICLE INFO

Article history:
Received 10 May 2013
Received in revised form
18 July 2013
Accepted 21 August 2013
Available online 25 September 2013

Keywords:
Antidepressant
Hypothalamus
Cytokine
Inflammation
Gene expression

ABSTRACT

To gain insight into the possible immune targets of antidepressant, we evaluated the expression of several inflammatory mediators in the hypothalamus of rats chronically (28 days) treated with the serotonin selective reuptake inhibitor fluoxetine (5 mg/kg, i.p.) or the tricyclic compound imipramine (15 mg/kg, i.p.). We focused our attention on the hypothalamus as it plays a key role in determining many of the somatic symptoms experienced by depressed patients. This brain region, critical also for expression of motivated behaviours, participates in the control of the hypothalamic-pituitary-adrenal axis activity and in stress response as well as coordinates physiological functions such as sleep and food intake that have been found altered in a high percentage of depressed patients. Notably, hypothalamus is a key structure for brain cytokine expression and function as it integrates signals from the neuro, immune, endocrine systems. By means of quantitative Real Time PCR experiments we demonstrated that a chronic treatment with either fluoxetine or imipramine resulted in a reduction of IL-6 and IFN-γ mRNAs and increased IL-4 mRNA expression in the rat hypothalamus. Moreover, we demonstrated that hypothalamic expression of members of IL-18 system was differentially affected by chronic antidepressant treatments. Chronically administered fluoxetine decreased IL-8 and CX3CL1 hypothalamic expression, while a chronic treatment with imipramine decreased p11 mRNA. Our data suggest that a shift in the balance of the inflammation toward an anti-inflammatory state in the hypothalamus may represent a common mechanism of action of both the chronic treatments with fluoxetine and imipramine.

1. Introduction

The anti-inflammatory effect of antidepressants (AD) has been the object of investigation for several decades (Abdel-Salam et al., 2003; Bianchi et al., 1994; Roumestan et al., 2007). ADs have been shown to decrease peripheral inflammation through central mechanisms in animal models of nociception and to possess an immunosuppressive effect as demostrated by *ex vivo* or *in vitro* approaches (Kubera et al., 2001; Maes et al., 2012; Obuchowicz et al., 2006). Increasing evidence is accumulating that demostrate the involvement of the immune system and in particular of their effectors, cytokines, in the development and progression of depression (Dantzer et al., 2008, 2011; Raedler, 2011). In particular, it is worth remembering how pro-inflammatory cytokines appear to be increased in blood or brain of patients with major depression (MD) and that pharmacological use of pro-inflammatory cytokines (i.e. interferon alpha) may induce MD (Alboni et al., 2013; Hepgul et al., 2012).

The hypothalamus represents an area of extreme interest because it integrates signals from the neuro, immune, endocrine systems. This area, because of its anatomical localization (Bennett, 2011) and the high density of cytokine receptors, seems to represent a key brain area to translate chemical message of cytokines into changes in endocrine, autonomic and behavioural functions. The hypothalamus may be involved in the development of the so-called neurovegetative symptoms of depression given its role in the control of food and water intake, sexual behaviour and reproduction and circadian rhythms. Moreover, this area is also an important centre for the integration of emotional impulse, motivation and cognition (Marchant et al., 2012; Rosen and Levenson, 2009; Simerly, 2004) and its transcriptome has been shown to be most affected by a classical antidepressant pharmacological treatment with respect to fast acting approaches such as sleep deprivation and electro convulsive therapy (Conti et al., 2007). The aim of this study was to explore whether a 28 day treatment with two of the most prescribed antidepressants, the tricyclic (TCA) imipramine and the selective serotonin reuptake inhibitor (SSRI) fluoxetine, was able to affect gene expression of targets involved in the inflammatory process in the rat hypothalamus.

By Real Time PCR we evaluated the effects of chronic treatments with either fluoxetine or imipramine on the expression of

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0014-2999/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2013.08.046

pro-inflammatory cytokines: Interleukin (IL-) 1β, Tumour Necrosis Factor (TNF) α, Interferon (IFN) γ, IL-6 and its receptor [IL-6R and gp130 (IL-6ST)], leukaemia inhibitory factor (LIF), IL-12a p35, IL-12b p40; IL-18 and its entire system [IL-18 binding protein (bp), IL-18 heterodimer receptor and the isoforms recently characterized in our laboratory (IL-18Rα type I, IL-18Rα type II, IL-18Rβ and IL-18Rβ short; Alboni et al., 2009, 2011a)]. Moreover, we assessed the effect of a 28-day treatment with ADs on the hypothalamic expression of a group of chemokines, a family of pro-inflammatory cytokines, including rat homologues of IL-8 (CXCL1 and CXCL2), its receptor CXCR2, fractalkine (CX3CL1) and its receptor CX3CR1 and two anti-inflammatory cytokines (IL-10, IL-4). Finally given their role in mediating AD response we also evaluated the ability of a chronic exposure to either fluoxetine or imipramine to affect the expression of the Brain-Derived Neurotrophic Factor (BDNF) and p11 (S100A10) mRNAs in the rat hypothalamus.

2. Material and methods

2.1. Animals

Experiments were performed on adult male Sprague-Dawley Albino rats of eight weeks of age at the beginning of the experimental procedure (Charles River, Calco, Italy). Male rats were used to avoid any effects of the oestrous cycle in female rats. Animals were housed 2 per cage in polycarbonate cages (28 × 17 × 12 cm) in a temperature- and humidity-controlled environment on a 12-h light-dark cycle (lights on at 6.00 a.m.) with ad libitum access to food and tap water. The procedures used in this study were in strict accordance with the 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. Drugs

Fluoxetine HCl (Polichimica), 5 mg/kg was dissolved in injectable sterile water at a volume of 1 ml/kg. Imipramine (Sigma Aldrich), 15 mg/kg was dissolved in injectable saline in a volume of 1 ml/kg body weight. All drugs were injected via an intraperitoneal (i.p.) route.

2.3. Pharmacological treatments

Animals ($n=8$ in all groups) were subjected to a chronic (28 days) treatment with fluoxetine (5 mg/kg/i.p.), imipramine (15 mg/kg/i.p.) or saline (1 ml/kg/i.p.). Doses were chosen in accordance with previous experiments performed in our laboratory and with previously published papers (Brunello et al., 2006; Nibuya et al., 1996; Vitale et al., 2009). Rats were sacrificed 18 h after the last injection; hypothalamus was dissected, immediately frozen on dry ice and stored at −80 °C until further analyses.

2.4. RNA extraction and retro transcription

Total RNA extraction and DNAse treatment were performed as previously described (Benatti et al., 2012). Two micrograms of total RNA was reverse transcribed with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) in 40 μL of reaction mix.

2.5. Real Time PCR

Real Time PCR was performed in ABI PRISM 7900 HT (Applied Biosystems) using Power SYBR Green mix (Applied Biosystems) as previously described (Benatti et al., 2012). Target mRNA levels were normalized for each well to endogenous control glyceraldehydes-3-phosphate dehydrogenase (GAPDH) (NCBI GenBank accession number: GAPDH mRNA: NM_017008.3).

Forward and reverse primers for the evaluated targets were used at the final concentration of 150 nM. The cycling parameters were 94 °C 2 min and 94 °C 15 s, 60 °C 1 min for 40 cycles. PCR products were subjected to a heat dissociation protocol (gradual increase of temperature from 60 °C to 95 °C) and agarose gel separation to verify the absence of artefacts, such as primer–dimers or non-specific products. Direct detection of PCR products was monitored by measuring an increase in fluorescence intensity caused by binding of SYBR GREEN I dye to neo-formed double strand DNA during the amplification phase. Ct (cycle threshold) value was determined by the SDS software 2.2.2 (Applied Biosystems) and was utilized to calculate mRNA fold changes using the delta delta ct (ΔΔCt) method. A brief description of the target genes and primers is listed in Table 1.

2.6. Statistical analysis

The mRNA levels were normalized to the endogenous control, GAPDH. Endogenous control mRNA levels were not affected by any treatment ($p > 0.05$, One-way ANOVA). For quantitative evaluation of changes the comparative ΔΔCt method was performed, using as calibrator the average levels of expression of saline control animals. Data were analyzed with a ONE-Way ANOVA. Planned pairwise post-hoc comparisons (Dunnett) with saline-treated animals were performed. All mean differences were considered statistically significant if $p < 0.05$. Analyses were conducted using SPSS for Windows® v.17 (SPSS Inc., Chicago, USA).

3. Results

3.1. A chronic treatment with either fluoxetine or imipramine results in an inhibition of pro-inflammatory cytokines expression in the rat hypothalamus

We first evaluated the expression levels of different pro-inflammatory cytokines that are proposed to be involved in depression and AD response: IL-1β, TNF-α, IFN-γ, IL-6 and IL-12 (Cattaneo et al., 2013; Dantzer et al., 2008; Maes et al., 2012; Miller et al., 2009; Zunszain et al., 2011).

A chronic treatment with either fluoxetine or imipramine failed to affect the expression of the pro-inflammatory cytokines IL-1β and TNF-α in the rat hypothalamus [One-way ANOVA $F(2;18)= 0.571$; $p > 0.05$ and $F(2;17)= 0.793$, $p > 0.05$, respectively; Fig. 1A and B]. Interestingly, One-way ANOVA revealed a main effect of a chronic antidepressant treatment for both IFN-γ and IL-6 hypothalamic mRNA levels. IFN-γ expression was significantly decreased in hypothalamus of animals receiving either fluoxetine or imipramine for 28 days with respect to their saline exposed counterparts [One-way ANOVA $F(2;17)= 7.776$; $p=0.006$; Fig. 1C]. A similar effect was observed for IL-6 mRNA levels: imipramine or fluoxetine administered chronically resulted in a significant decrease of hypothalamic expression levels of this cytokine with respect to saline-receiving control animals [One-way ANOVA $F(2;17)= 6.666$; $p=0.007$; Fig. 1D].

To further explore the effect of a chronic treatment with antidepressants of two different classes on IL-6 system, we focused on two molecules necessary for this cytokine to exert its activity: IL-6