Hormones and Behavior 73 (2015) 64–74

Contents lists available at ScienceDirect

Hormones and Behavior

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

Astrocytic IL-6 mediates locomotor activity, exploration, anxiety, learning and social behavior

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ARTICLE INFO

Article history:
Received 13 January 2015
Revised 7 May 2015
Accepted 25 June 2015
Available online 2 July 2015

Keywords:
Anxiety
Astrocyte
Elevated plus-maze
Exploratory behavior
Hole-board
IL-6
Intrauterine survival
Spatial learning
Morris water-maze
Transgenic mice

ABSTRACT

Interleukin-6 (IL-6) is a major cytokine in the central nervous system, secreted by different brain cells and with roles in a number of physiological functions. We herewith confirm and expand the importance of astrocytic production of and response to IL-6 by using transgenic mice deficient in astrocytic IL-6 (Ast-IL-6 KO) or in its receptor (Ast-IL-6R KO) in full C57Bl/6 genetic background. A major prosurvival effect of astrocytic IL-6 at early ages was clearly demonstrated. Robust effects were also evident in the control of activity and anxiety in the hole-board and elevated plus-maze, and in spatial learning in the Morris water-maze. The results also suggest an inhibitory role of IL-6 in the mechanism controlling the consolidation of hippocampus-dependent spatial learning. Less robust effects of astrocytic IL-6 system were also observed in despair behavior in the tail suspension test, and social behavior in the dominance and resident–intruder tests. The behavioral phenotype was highly dependent on age and/or sex in some cases. The phenotype of Ast-IL-6R KO mice mimicked only partially that of Ast-IL-6KO mice, which indicates both a role of astrocytes in behavior and the participation of other cells besides astrocytes. No evidences of altered function of the hypothalamic–pituitary–adrenal axis were observed. These results demonstrate that astrocytic IL-6 (acting at least partially in astrocytes) regulates normal behavior in mice.

Introduction

Interleukin 6 (IL-6) is a plurifunctional four-helix bundle cytokine, originally identified as a B-cell differentiation factor in 1985 (Hirano et al., 1985), which has been linked to numerous biological functions over these years but particularly in the central nervous system (CNS) (Erta et al., 2012; Gruol and Nelson, 1997; Spooren et al., 2011).

Besides the control of body weight and temperature, stress response, neurogenesis and several other physiological variables, IL-6 is involved in the control of a number of behavioral traits. Our group previously demonstrated that total IL-6 KO mice (Kopf et al., 1994) had an increased emotional reactivity in novel environments (hole-board and elevated plus-maze tests), exhibiting less deambulation, rearings and head-dipping behavior in the hole-board test and less exploration of the open arms (thus increased anxiety) in the elevated plus-maze test (Armario et al., 1998). However, this does not seem to be a very robust finding. Another group confirmed the decrease in rearings and the increase in anxiety using the same IL-6 KO mice, but these authors found opposite results regarding locomotion (Butterweck et al., 2003). Other authors observed altered exploration and anxiety levels of IL-6 KO mice depending on the specific settings (Baier et al., 2009; Butterweck et al., 2003), or genetic background (Swiergiel and Dunn, 2006).

Other behavioral parameters under discussion are those related to learning and memory. Exogenous administration of IL-6 leads to impaired spatial learning in rats (Samuelsson et al., 2006), but IL-6 deficiency in IL-6 KO mice was also reported to impair learning in both hippocampus-independent (novel object recognition memory test) and dependent learning (Morris water maze test) by some authors (Baier et al., 2009), although others found no changes (Sparkman et al., 2006) or even a better learning performance in IL-6 KO mice in radial maze learning (Braida et al., 2004).

Cell-specific production of IL-6 might have a role in this disparity of results. For example, transgenic expression of astrocyte-derived IL-6 in mice (GFAP-IL-6 mice) has been shown to cause severe neurological disease (Campbell et al., 1993) and a progressive decline in avoidance learning (Heyser et al., 1997), while transgenic expression of neuronal IL-6 produced no neuronal deficits (Fattori et al., 1995), but caused opposite alterations in social behavior compared to the ones described for GFAP-IL-6 mice (Alleva et al., 1998). The fact that IL-6 can eventually be produced by neurons, astrocytes, microglia and endothelial cells, and that in turn these cells can respond to IL-6, may be a significant factor for these discrepancies. Thus, the role of IL-6 should be studied

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http://dx.doi.org/10.1016/j.yhbeh.2015.06.016
0018-506X/

in a cell-specific fashion. Astrocytes are major responders to IL-6 as well as one of its main CNS producers. We previously hypothesized and demonstrated that astrocytic IL-6 deficiency (as well as astrocytic IL-6 receptor deficiency) had a role in some physiological and behavioral variables like locomotion, exploration and anxiety (Quintana et al., 2013). In this study we have expanded these initial results by using mice backcrossed at least 10 times with C57BL/6 mice, both male and female mice, of different ages, and a bigger battery of behavioral tests. Therefore, besides studying locomotion and exploratory behavior and anxiety, we have characterized for the first time the role of astrocyte-derived IL-6 and astrocytic IL-6 receptor deficiency in spatial learning, despair behavior and social behavior.

Materials and methods

Animals

Mice were maintained with food (Harlan global diet 2918) and water available ad libitum under standard conditions. All experiments performed were approved by the UAB Animal Care Committee. IL-6 floxed mice were generated by our group as described elsewhere (Quintana et al., 2013) and backcrossed with C57BL/6 mice for at least 10 generations. IL-6R floxed mice were generously provided by Dr. Angela Drew, generated as described elsewhere (McFarland-Mancini et al., 2010), and glial fibrillary acidic protein (GFAP) promoter-specific Cre recombinase-expressing mice (GFAP-Cre mice, 01XN3) were obtained from the National Cancer Institute Mouse Models of Human Cancers Consortium (MMHCC) at Frederick, MD 21702, USA. Astrocyte IL-6 KO (Ast-IL-6 KO) and Astrocyte IL-6 Receptor KO (Ast-IL-6R KO) mice were obtained as previously described (Quintana et al., 2013). Three month-old CD-1 Swiss male mice were obtained from Harlan and were kindly gifted by Cristina Rabasa and Antonio Armario.

Battery of behavioral tests

Mice of the Ast-IL-6 cross were studied at two different ages: ~4 week-old and ~8 month-old at the beginning of the battery of tests. At these ages the mice were transferred to the colony testing room for handling and habituation during at least one week as previously described (Quintana et al., 2013). The mice were sequentially tested in the hole-board, elevated plus-maze, tail suspension, Morris water-maze, dominance tube and resident–intruder tests as outlined in Supplementary Fig. 1. Mice of the Ast-IL-6R cross were studied at ~3–4 months of age. Experiments were recorded with a video camera recorder (Everio G camera, JVC) and the test lighting area was approximately 90 lx. All behavioral tests were carried out and analyzed blindly.

Hole-board test (HB)

HB was originally devised by Boissier and Simon (Boissier and Simon, 1962); it allows to separately asses locomotion and exploration (measured by Head-dipping behavior) in mice confronted with a new environment. HB was carried out as described previously (Quintana et al., 2013). Total, external and internal ambulation, rearings, defecations and frequency and duration of head-dipping events were assessed. Locomotion and rearings were measured by visualizing the recorded video with a self-developed Excel program (Quintana et al., 2013).

Elevated plus-maze test (EPM)

EPM, originally devised by File et al. (Pellow et al., 1985), is a widely used rodent model for characterizing anxiety. EPM was carried out as described previously (Quintana et al., 2013). Three or seven days (depending on age group) after performing the HB test, animals were subjected to the EPM during the first part of the light cycle (8 to 12 AM.). Number of entries and time spent in both open and closed arms (both absolute and as a percentage of total arm entries or total time spent in the arms); time spent in the central square, latency of central square first escape, locomotion (crossed squares), number of defecations, number of rearings and number of protected, unprotected and total head-dips, were analyzed from the videos by using a self-developed application for Excel (Quintana et al., 2013).

Tail suspension test (TST)

TST is a simple screening test for either the behavioral effects of antidepressants or the individual differences in stress responses in rodents. It is based on the observation that after initial escape-oriented movements, mice develop an immobile posture when placed in an inescapable stressful situation (being hung by their tail) (Cryan et al., 2005). This behavioral test was performed between 2:00 and 6:00 p.m. Mice were suspended by the tail (with adhesive tape) in the TST apparatus. The test lasted 6 min and results were analyzed in three 2-minute periods.

Morris water maze test (MWM)

MWM is widely used for studying spatial memory and learning in rodents, relying on distal cues to navigate from random start locations around the perimeter of an open swimming area to locate a submerged escape platform. Spatial learning is assessed across repeated trials and reference memory is determined by preference for the platform area when the platform is absent. Different mechanisms of learning and memory can be tested in this maze using different training protocols (Vorhees and Williams, 2006). Our MWM tank consists of a propylene round pool (120 cm diameter × 60 cm deep), which is virtually divided in 4 equal quadrants, with a removable platform that can be adjusted at different heights (8 cm diameter). It contains water at 22 ± 1 °C made opaque by the addition of approximately 1 cl/l non-toxic liquid latex (Látex Compound Española S.A). Four black cues (12 × 12 cm) and four white cues (24 × 24 cm) were placed in the pool wall of each quadrant and in a black curtain surrounding the pool, respectively (Supplementary Fig. 6). It was performed between 2:00 and 6:00 p.m. Except for the probe trials, mice were always given 60 s to swim and reach the platform and then allowed to remain on it for 15 s. Mice unable to find it in that time were guided to and allowed to remain on it for 15 s. Trials were separated by 60–80 min; during intervals mice were dried with paper and kept into their cages in the test room. We tested the following paradigms.

Visible platform test/cued learning test (Day 1)

This is a control condition that consists in testing the ability to learn to swim to a cued goal. The platform was elevated above the water surface and a visible black 'flag' was mounted on it making it easily identifiable from across the pool. To ensure that animals were using this proximal cue to locate the platform, both the location of the goal and the starting point were moved to new positions in each trial.

Hidden platform test (Days 2–5)

Mice were challenged to escape from the pool by finding a hidden platform (submerged 1 cm below opaque water) using distal cues as spatial references. It is the most basic MWM procedure. The platform was on a fixed location relative to the cues across days and mice were placed into the water facing the edge of the pool at semi-random start positions across trials (4 trials per day). Animals were trained for 4 consecutive days and the latency to find the hidden platform in each trial was recorded.

Probe trial test (Days 5–6)

To assess reference memory at the end of learning, a probe trial (without the platform) was given. We gave each animal two probe