Available online at www.sciencedirect.com
SCIENCE DIRECT
Molecular Brain Research 137 (2005) 159–165
MOLECULAR BRAIN RESEARCH
www.elsevier.com/locate/molbrainres

Research report

Cytokine mRNA levels in brain and peripheral tissues of the rat: Relationships with plus-maze behavior

Cornelius R. Pawlak[a,*], Rainer K.W. Schwarting[a], Artur Bauhofer[b]

[a]*Experimental and Physiological Psychology, Philipps-University of Marburg, Gutenbergstr. 18, 35032 Marburg, Germany*
[b]*Institute of Theoretical Surgery, Philipps-University of Marburg, 35043 Marburg, Germany*

Accepted 3 March 2005
Available online 12 April 2005

Abstract

There is evidence that interleukin (IL)-2 may be related to anxiety as measured in the elevated plus-maze. Recently, we showed that normal adult male Wistar rats can differ systematically in this test of avoidance behavior, that is, time spent on the open arms of the elevated plus-maze. Rats with low open arm time had higher striatal levels of IL-2 mRNA than those with high open arm time, but did not differ significantly in expression of other striatal cytokine mRNA. Here, we investigated whether these expression effects are anatomically specific to the striatum. Therefore, we asked in this double-blind study whether elevated plus-maze behavior may also be related to endogenous levels of cytokine mRNA in other brain regions, which play a role for anxiety, namely the amygdala, hippocampus, and the prefrontal cortex. Additionally, and as peripheral controls, immuno-neuro-endocrine relevant tissues (adrenal glands, spleen) were analyzed. Based on open arm time in the elevated plus-maze, male Wistar rats were divided into sub-groups with either low or high open arm time behavior. Then, IL-1β, IL-2, IL-6, and tumor necrosis factor (TNF)-α cDNA levels were measured post-mortem using semi-quantitative, competitive, reverse transcription polymerase chain reaction. First, we found that cytokine expressions differed considerably between and within these central and peripheral tissues. Secondly, rats with high compared to low open arm time behavior showed higher IL-2 mRNA levels in the prefrontal cortex, which is an inverse pattern to what we recently found in the striatum. These results provide new evidence indicating that cytokine mRNA in the brain can be related to elevated plus-maze behavior and that this relationship is site (prefrontal cortex, striatum)- and cytokine mRNA-specific (IL-2).

Theme: Neural basis of behavior
Topic: Motivation and emotion

Keywords: Interleukin-2; Interleukin-1; Interleukin-6; TNF-α; Prefrontal cortex; Striatum; Hippocampus; Amygdala; Spleen; Adrenal glands; Anxiety; Avoidance behavior; Elevated plus-maze

1. Introduction

The elevated plus-maze (EPM) is a widely used behavioral paradigm which measures unconditioned avoidance behavior and which has been extensively assessed as a test for anxiety-like behavior in rats [17]. During a typical EPM test, animals will actively avoid the open arms in favor of the closed arms. Work with selectively bred Wistar rats [15] and normal outbred Wistar rats [16] has shown that such animals can differ systematically in open arm time behavior in the EPM. For example, outbred rats of the same strain, sex, and age can be characterized as high (HOA) and low open arm (LOA) rats based on their time spent on the open arms [35]. Such individual differences in plus-maze behavior seem to reflect a trait or behavioral disposition which is distinct from that observed in the open field [34]. Physiologically, we found this trait to be related to the neurotransmitter serotonin in the ventral striatum [35], a brain region which is critical for motivated behavior, and a transmitter which is critical for anxiety [10]. Recently, we

* Corresponding author. Fax: +49 6421 2823610.
E-mail address: pawlak@staff.uni-marburg.de (C.R. Pawlak).

0169-328X/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.molbrainres.2005.03.002

have also shown that LOA (then termed HA) rats had higher striatal levels of IL-2 mRNA compared to HOA (then termed LA) rats, but did not differ significantly in expression of other striatal cytokine mRNA levels [28]. Interestingly, there is evidence that cytokines may influence the release of serotonin in the ventral striatum and that the profile of changes is cytokine-specific [39].

Cytokines have been shown to affect behavior, e.g., IL-2 induces behavioral changes in novelty-induced locomotion [42], hedonic processes [24], and spatial memory [22]. Only few studies have analyzed anxiety-like behavior, and IL-2, with yet inconsistent results. In patients with anxiety disorders, decreased IL-2 production was observed peripherally [20]. However, in animal models for anxiety, neither acute nor repeated IL-2 administration effectively influenced anxiety-like behavior (plus-maze or neophobia) [1,6,22,30]. It is also unknown whether endogenous cytokines in specific brain areas may be related to avoidance behavior. Interestingly, however, IL-2/15Rβ knockout mice compared to wild-type and heterozygote mice exhibited decreased levels of closed arm time in the EPM [31].

Here, we extended our previous work [26] and investigated whether differences in cytokine mRNA levels between HOA and LOA rats may also occur in tissues other than the striatum. We performed this analysis in the same animals, from which we had obtained the striatal data before [28]. Here, we examined other brain areas (hippocampus, amygdaloid cortex, prefrontal cortex) which play a role for anxiety, especially in relation to serotonin [14,19,26]. Furthermore, two peripheral tissues involved in the immuno-neuro-endocrine system (adrenal glands, spleen) were analyzed in comparison to test whether cytokine expressions in such rats may differ also at the peripheral level. Based on the percentage of open arm time in the EPM, male adult Wistar rats were divided into HOA and LOA sub-groups [16,28]. Then, IL-1β, IL-2, IL-6, and tumor necrosis factor (TNF)-α cDNA levels were measured post-mortem in brain and control peripheral tissues.

2. Materials and methods

2.1. Animals

Thirty-four male outbred Wistar Unilever rats (Harlan Winkelmann, Borchen, Germany) weighing 270–330 g at the beginning of the experiment were used. All animals analyzed in the present study were identical to those reported in Pawlak et al. [28]. They were housed in groups of five per cage (cage size: length 57 × width 35 × height 24 cm) under standard laboratory conditions with a standard diet and tap water ad libitum (containing 0.0004% hydrochloric acid to avoid contamination). The housing room was maintained on a 12-h light/dark cycle (lights on: 7:00–19:00 h). Ambient temperature was 23 ± 1 °C. The study was conducted in the light cycle (10:00–16:00 h). Four days upon arrival in the laboratory, all animals underwent gentling and handling daily (5 min each) for 3 days prior to behavioral testing. Experiments were conducted in accordance with the ethical regulations for animal experimentation at the University of Marburg.

2.2. Elevated plus-maze

According to our routine procedure [16], the animals were first exposed to an open field on two consecutive days (10 min each; data not shown). Four days after the last open field test, the rats were tested once (5 min) in the EPM (50 cm above the floor), which was illuminated by four red bulbs (28 lx in the center). The EPM was made of plastic and consisted of two opposite open arms (50 × 10 cm), two opposite closed arms (50 × 10 cm; with 40 cm high walls), and a middle section (10 × 10 cm). Behavior was recorded by video. The following behavioral measures were scored from videotape by observation: (1) the time spent on open arms; (2) the number of entries into all arms; (3) the number of entries into closed arms; (4) the number of rearings. Based on the percentage of time spent on the open arms relative to total arm time (open arm time/total arm time × 100), the animals were divided into HOA (n = 17) and LOA (n = 17) rats by median split [16,28,35].

2.3. Probe extraction

Twenty-four hours after EPM testing, all animals were anesthetized with sodium pentobarbital (Narcoren®; Merial GmbH, Hallbergmoos, Germany, 1.5 ml/kg, ip), decapitated, and both brain hemispheres were quickly removed freehand. Immediately after excision, the hippocampus, amygdaloid cortex, prefrontal cortex, adrenal glands, and spleen tissue samples were weighed and frozen in liquid nitrogen.

2.4. RNA analyses

Until RNA extraction with RNA-Clean® (ASG, Heidelberg, Germany), the samples were stored at −70 °C. Extracted RNA was stabilized with 40 U/μl RNasin® (Promega, Madison, USA). For semi-quantitative, competitive reverse transcription polymerase chain reaction (RT-PCR), a multispecific competitor fragment for rat cytokines was used [38]. Before amplification of IL-1β, IL-2, IL-6, and TNF-α, the housekeeping gene β-actin was amplified to check the efficiency of the reverse transcriptase reaction. When needed, a correction was introduced in order to start with the same amount of cDNA in each tube (cDNA equivalent of about 5 ng total RNA). After PCR, the samples were separated in a 1% agarose gel, ethidium bromide stained, digitized, and analyzed with the Gelscan Software (BioSciTec, Marburg, Germany). For quantification, only bands with similar intensity on the gray scale between the competitor fragment and the cytokine of interest were used.