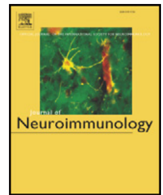
Journal of Neuroimmunology 257 (2013) 59–66

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

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



Neonatal stress modulates sickness behavior: Role for proinflammatory cytokines

Ronit Avitsur [a,*], Rachel Maayan [b], Abraham Weizman [b,c]

[a] School of Behavioral Sciences, The Academic College of Tel Aviv-Yaffo, Yaffo, Israel
[b] The Laboratory of Biological Psychiatry, Felsenstein Medical Research Center, Beilinson Campus, Sackler Faculty of Medicine, Tel Aviv University, Petah Tikva 49100, Israel
[c] Research Unit, Geha Mental Health Center, Petah Tikva, Israel

ARTICLE INFO

Article history:
Received 18 October 2012
Received in revised form 22 January 2013
Accepted 14 February 2013

Keywords:
Maternal separation
Sickness behavior
Lipopolysaccharide
Interleukin-1
Tumor necrosis factor-α
Corticosterone

ABSTRACT

Neonatal stress increased the duration and augmented symptoms of sickness behavior induced by influenza virus infection or endotoxin challenge in mice. Since proinflammatory cytokines were implicated in sickness behavior, the present study sought to determine the effect of neonatal stress on cytokines-induced sickness behavior and on proinflammatory cytokine secretion. Data indicate that separation of mouse pups from the dams at an early age (maternal separation, MSP) increased the duration and augmented some of the symptoms of sickness behavior induced by proinflammatory cytokines. In addition, MSP partially suppressed cytokine and corticosterone secretion in response to endotoxin administration. These data may suggest that MSP increased sensitivity to the effects of proinflammatory cytokines on sickness behavior following an immune challenge.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Early stress has long been known to alter multiple physiological systems, consequently affecting early development of neuroendocrine and neuroimmune systems (Ackerman et al., 1988; Plotsky and Meaney, 1993; Neveu et al., 1994; Suchecki and Tufik, 1997; Francis and Meaney, 1999; Gutman and Nemeroff, 2002; Teunis et al., 2002; Coe and Lubach, 2007; Kruschinski et al., 2008; Meagher et al., 2010). In an animal model of neonatal stress, rat or mouse pups were repeatedly separated from the dam during critical periods of their development (maternal separation, MSP). Studies have shown that MSP altered immune activity and increased vulnerability to disease. For example, MSP reduced resistance to viral and parasitic infection (Avitsur et al., 2006; Barreau et al., 2006; Meagher et al., 2010), increased severity of colitis, with increased production of proinflammatory cytokines (Ghia et al., 2008; Faturi et al., 2010) and augmented tumor colonization in the presence of chronic restraint stress (Nakamura et al., 2011).

Febrile infectious diseases are typically accompanied by a behavioral syndrome termed "sickness behavior" (Hart, 1988; Kent et al., 1992), including reduced locomotion and exploratory activity, anorexia and anhedonia, and reduced libido (Avitsur and Yirmiya, 1999b; Dantzer et al., 2007). Sickness behavior may be a strategy important for the survival of the individual, in that it promotes energy conservation and reduces the risk of encountering predators (Hart, 1988; Kent et al., 1992). Interestingly, MSP affected the kinetics and severity of sickness behavior in mice (Avitsur and Sheridan, 2009). Following a mild infection with influenza virus, reduced food consumption was observed in control females on day 7 post infection, whereas in MSP females reduced food consumption was observed earlier – on day 6 post infection, and lasted longer – until day 9 post infection. In infected males, reduced food consumption and reduced saccharin consumption were significantly more severe in MSP mice compared to controls. Similarly, in endotoxin exposed mice, reduced food consumption and body weight loss appeared earlier in MSP compared to control mice resulting in longer duration of sickness symptoms in MSP mice (Avitsur and Sheridan, 2009). Together these findings suggested that neonatal stress had long lasting effects on the behavioral response to influenza virus infection and endotoxin administration (Avitsur et al., 2006; Avitsur and Sheridan, 2009).

Endotoxin administration activates the innate immune response. Mononuclear cells release the proinflammatory cytokines interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α during the first 30–90 min after exposure and in turn activate a second level of inflammatory cascades, including cytokines, lipid mediators and reactive oxygen species, as well as up-regulating cell adhesion molecules (Cohen, 2002). Proinflammatory cytokines secreted as a part of this inflammatory process were found to induce sickness behavior (Dantzer et al., 2007). In a similar manner, influenza virus infection in mice induced the expression of proinflammatory cytokine secretion in the infected lungs, suggesting a similar role for proinflammatory cytokines in influenza virus-induced sickness behavior (Dantzer et al., 2007). Studies have further shown that glucocorticoids secreted as a part of the inflammatory process played a protective role in limiting the expression of the

* Corresponding author at: School of Behavioral Sciences, The Academic College of Tel Aviv-Yaffo, 14 Rabenu Yerucham St., PO Box 8401, Yaffo 68114, Israel. Tel.: +972 3 680 2547; fax: +972 3 680 2526.
E-mail address: avitsur@mta.ac.il (R. Avitsur).

0165-5728/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jneuroim.2013.02.009

behavioral symptoms of illness (Johnson et al., 1996). In support of these findings, an additional study showed that MSP elevated gene expression and protein synthesis of lung proinflammatory cytokines and suppressed circulating corticosterone levels in influenza virus infected mice (Avitsur et al., 2006). Together, these findings suggest that neonatal stress may have implications for host response to an invading pathogen.

To sum up, studies have shown that MSP modulated sickness behavior induced by influenza virus infection and endotoxin administration (Avitsur and Sheridan, 2009). Further reports implicated proinflammatory cytokines in mediating sickness behavior following immune activation (Dantzer et al., 2007). Based on these findings, the present study sought to examine the effects of MSP on sickness behavior induced by proinflammatory cytokines and on endotoxin-induced proinflammatory cytokine secretion in mice.

2. Materials and methods

2.1. Animals

Subjects were offspring (females and males) of C57BL/6 mice purchased from Harlan Laboratories (Israel). Subjects were born and raised at the Academic College of Tel Aviv-Yaffo animal facility. All subjects were given free access to food and water and were maintained on a reversed 12-hour light/dark cycle (lights on at 7:00 PM). Animal care procedures were approved by the National Israeli Committee of Animal Care and Use. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available.

2.2. Maternal separation (MSP)

MSP procedure was described previously (Avitsur et al., 2006; Avitsur and Sheridan, 2009). Breeding and MSP were conducted in standard housing cages. Male and female C57BL/6 breeder mice were housed together for a week (1 male with 2 females), after which they were separated to individual cages. The age of the pups was determined by checking for births daily, the date of birth was designated as postnatal day 0. Litters were housed with the dam and cages were not disturbed except for MSP procedure. MSP consisted of removing the pups from the home-cage and into a clean standard laboratory cage in sibling groups, while the dam remained in the home cage. MSP was performed for 6 h a day between postnatal days 1 and 14. The cages with the pups were placed on a heating pad set at 30–33 °C. Pups had no access to food and water during the separation period. Following separation pups were returned into the home cages. Close observations were conducted during separation and following the return of the pups into their home cages. Observations indicated that in most cases (>99%) pups did not look weak or debilitated during or following separation. All pups were retrieved into the nest by the dams within 2–3 min from their return to their home cage. Control litters remained undisturbed in their home cages. Following weaning (at postnatal day 24–26), mice were housed in same-sex groups with their littermates. In all experiments, up to 2 pups per litter were included in the same treatment group.

2.3. Cytokine and LPS administration

Cytokines (ProSpec-Tany TechnoGene Ltd., Enco Scientific Services, Israel) and Lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 (Sigma, Israel) were injected ip in 0.2 ml sterile saline. Control mice were administered with 0.2 ml sterile saline, ip. Recombinant mouse (rm) IL-1β was administered at 0.1 μg/mouse. RmTNFα was administered at 1 μg/mouse. LPS was administered at 1 or 5 μg/mouse. Doses of all drugs were selected based on preliminary studies showing mild, relatively short-lasting effects in conditions similar to those used in the present study.

2.4. Cytokines and hormone assessment

2.4.1. Tumor Necrosis Factor alpha (TNF-α)

Serum level of TNF-α were assayed using Quantikine mouse TNF-α immunoassay solid phase ELISA Kit (R&D Systems, Minneapolis, USA). Precision: intra-assay 4.3–9%, inter-assay 6.1–9.2% according to level. Sensitivity 5.1 pg/ml; Specificity: cross-reactivity with rat TNF- α – 78%, all others – negligible; range of detection 23.4–1500 pg/ml (values obtained from manufacturer).

2.4.2. Interleukin-1 beta (IL-1β)

Serum IL-1β levels were assayed using Quantikine mouse IL-1β immunoassay solid phase ELISA Kit (R&D Systems, Minneapolis, USA). Precision: intra-assay 1.5–4.4%, inter-assay 2.8–6.1% according to level. Sensitivity 3.0 pg/ml; Specificity: no significant cross-reactivity was observed excluding human IL-1β (1%) rat IL-1β (4%); range of detection 7.8–500 pg/ml (values obtained from manufacturer).

2.4.3. Interleukin-6 (IL-6)

Serum IL-6 levels were assessed using Quantikine mouse IL-6 immunoassay solid phase ELISA Kit (R&D Systems, Minneapolis, USA). Precision: intra-assay 3.5–7.0%, inter-assay 6.1–8.9% according to level. Sensitivity 1.6 pg/ml; Specificity: no significant cross-reactivity was observed excluding rat IL-6 (0.1%); range of detection 7.8–500 pg/ml (values obtained from manufacturer).

2.4.4. Corticosterone

Serum corticosterone levels were assessed using Corticosterone ImmuChem™ Double Antibody radioimmunoassay (RIA) kit (MP Biomedicals LLC, Orangeburg, NY, USA). Precision: intra-assay 4.4–10.3% (within runs), inter-assay 6.5–7.2% (between runs) according to level; Sensitivity: minimal detectable dose 7.7 ng/ml; Specificity: cross-reactivity with similar steroids — negligible; range of detection 25–1000 ng/ml (values obtained from manufacturer).

2.5. Procedure

2.5.1. Experiment 1: effect of MSP on cytokine-induced sickness behavior

Mouse pups underwent control or MSP manipulations as described. Further experimental manipulations took place when subjects reached the age of 7–10 weeks. At least 48 h before the beginning of baseline measurements, subjects were individually housed. Water bottles in each cage were replaced with bottle of a sucrose solution (2% in dH2O, Sigma) for a period of 24 h, followed by a period of at least 24 h with no exposure to sucrose (water only). For baseline measurements mice were weighed, returned to their home cage and provided with weighed food, and two drinking bottles: water and sucrose. Food pellets and drinking bottles were weighed again 2, 4, 6 and 24 h later. Following the last baseline measurements, animals were weighed and injected with either saline or a cytokine (IL-1β in *experiment 1a* and TNFα in *experiment 1b*). Animals were then supplied with fresh weighed food and drinking bottles. Body weight, food and drinking bottles were weighed again 2, 4, 6 and 24 h following injections. Occasionally a bottle leaked; when this occurred, data for that liquid at that time point were discarded. The different cytokines (IL-1β or TNFα) were tested in separate experiment using different groups of mice. An additional study was conducted in order to examine the effect of MSP on the response to IL-6 administration. However, administration of up to 0.5 microgram/mouse of IL-6 had no effect on body weight, food or fluid consumption in control and MSP males and females ($p>0.1$, see also Hawkley et al., 2007), thus data describing the response to IL-6 administration are not presented. The design of this study required that experiments will be broken down to multiple repeats. Thus,

Download English Version:

https://daneshyari.com/en/article/6020707

Download Persian Version:

https://daneshyari.com/article/6020707

Daneshyari.com