Available online at www.sciencedirect.com
ScienceDirect
Brain, Behavior, and Immunity 22 (2008) 838–849
BRAIN, BEHAVIOR, and IMMUNITY
www.elsevier.com/locate/ybrbi

Interleukin (IL)-6 and IL-1β act synergistically within the brain to induce sickness behavior and fever in rats

Lois M. Harden [a,*], Irné du Plessis [a], Stephen Poole [b], Helen P. Laburn [a]

[a] *Brain Function Research Group, School of Physiology, Medical School, University of the Witwatersrand, 7 York Road, Parktown, Gauteng, Johannesburg, South Africa*
[b] *Division of Immunology and Endocrinology, National Institute for Biological Standards and Control, Potters Bar, Herts, UK*

Received 19 September 2007; received in revised form 22 November 2007; accepted 23 December 2007
Available online 5 February 2008

Abstract

Pro-inflammatory cytokines interleukin (IL)-6 and IL-1β can act in the brain (centrally) to cause fever. Sickness behaviors which accompany fever also appear to involve the central action of IL-1β. We injected species-homologous rat IL-6 and IL-1β directly into the brains of conscious rats to examine the effect of these cytokines on fever, and two behaviors affected by sickness, voluntary wheel-running and food intake. Male Sprague–Dawley rats selected for their predisposition to spontaneously run on running wheels were used in the experiment. Each rat was anaesthetized and had a temperature-sensitive radiotransmitter implanted intra-abdominally, and a 23-gauge stainless steel guide cannula inserted stereotaxically over the lateral cerebral ventricle. Rats were randomly assigned to receive intracerebroventricular injections of three doses of either IL-1β or IL-6 (100 ng, 1 ng or 0.1 ng IL-1β and 200 ng, 20 ng or 2 ng IL-6), or one of three different combinations of IL-1β and IL-6. Rats receiving either IL-1β or IL-6 showed a dose-dependent increase in body temperature and decrease in wheel-running (ANOVA, $p < 0.0001$). Only rats receiving the highest dose of IL-1β significantly decreased food intake and body mass compared to rats receiving vehicle (ANOVA, $p < 0.001$). Doses of IL-1β and IL-6 which, when injected on their own were non-pyrogenic and did not affect food intake and body mass, induced fever and anorexia when they were co-injected centrally. These results show that species-homologous rat IL-6 and IL-1β can act directly within the brain to decrease voluntary activity and suggest they also can act synergistically to induce anorexia and fever.

Keywords: Voluntary wheel-running; Anorexia; Cachexia; Pro-inflammatory cytokines; Synergy

1. Introduction

Various brain-mediated behavioral responses, more commonly referred to as sickness behaviors, are observed together with the fever response in both animals and humans during an acute infectious illness (Hart, 1988; Dantzer, 2001). Some of the behavioral changes typically observed in sick individuals and animals include a loss of appetite and interest in social activities, increased sleep and depressed activity (Dantzer, 2001). Of these behavioral changes one of the most commonly reported by patients in various disease states or observed in infected animals, is fatigue accompanied by a decrease in daily activity (Ottenweller et al., 1998; Johnson, 2002; Hewlett et al., 2005; Kramer et al., 2005; Schubert et al., 2007).

Activity is routinely assessed in experimental animals by measuring voluntary wheel-running and recently we and others have shown that voluntary wheel-running in mice and rats is markedly reduced during infection (Sheng et al., 1996; Ottenweller et al., 1998; Sherwin, 1998; Katafuchi et al., 2003; Harden et al., 2006). Results from a study conducted in our laboratory investigating the functional importance of endogenous interleukin (IL)-6 in lipopolysaccharide (LPS)-induced sickness behavior, have shown that peripherally released IL-6 only partially mediates the suppression of voluntary activity induced by LPS administration (Harden

* Corresponding author. Fax: +27 11 643 2765.
E-mail address: Lois.Harden@wits.ac.za (L.M. Harden).

0889-1591/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2007.12.006

et al., 2006). However, administration of human recombinant IL-6 induces a sensation of fatigue in healthy humans at rest and decreases athletic performance in trained runners; treatment with IL-6 antibodies induces an immediate disappearance of previously debilitating fatigue reported by patients with multicentric Castleman disease, a disease characterized by a dysregulated overproduction of IL-6 (Spath-Schwalbe et al., 1998; Nishimoto et al., 2000; Robson-Ansley et al., 2004; Nishimoto et al., 2005). These data, obtained from animal and human studies therefore suggest some involvement of IL-6 in mediating sickness behavior responses such as fatigue and the associated reduction in physical activity thought to be triggered by the brain during infection. Nevertheless, to date it has been difficult to elicit sickness behaviors by central administration of IL-6 (Oitzl et al., 1993; Lenczowski et al., 1999).

In contrast to the lack of evidence for the direct involvement of IL-6 within the brain in mediating sickness behavior, there is substantial evidence that another pro-inflammatory cytokine, IL-1β, acts centrally to mediate sickness behavior. Central administration of IL-1β reproduces symptoms of sickness which include decreased food intake, body mass and locomotor activity (Anforth et al., 1998; Nadjar et al., 2005; Carmichael et al., 2006; Pecchi et al., 2006; Elander et al., 2007). These findings would tend to suggest that IL-1β is the more likely mediator of fatigue and associated reduction in physical activity observed during infection.

Cytokines probably do not function independently to induce host responses to infection, but rather they interact with other cytokines. In particular, cytokine interactions observed as physiological synergy in which the effect of a combination of substances exceeds the effect of the individual constituents, has been demonstrated *in vivo* between IL-1β and IL-6 (Lenczowski et al., 1999; Cartmell et al., 2000).

To discern direct effects in the brain of IL-6 and IL-1β in mediating sickness behaviors, we have injected species-homologous rat IL-6 and IL-1β directly into the brains of conscious rats and have examined the dose–response effects on voluntary activity, that is, voluntary wheel-running and food intake. We also measured body temperature to determine whether the effects of IL-6 and IL-1β on voluntary activity and feeding were secondary to fever. Moreover, we further assessed the possible physiological synergy between central IL-6 and IL-1β in mediating changes in behavior and body temperature by co-injection of the cytokines. Our results uncover a role for both central IL-6 and central IL-1β in mediating suppression of voluntary activity, and identify a synergistic effect of IL-6 and IL-1β in inducing anorexia and fever.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (initial body mass 120–150 g) were housed individually in cages to which exercise-training wheels had been attached. The rats were kept at an ambient temperature of 21 ± 2 °C and on a 12 h:12 h light:dark cycle (lights on at 07:00). Rats with an average voluntary daily running distance of 1 km, monitored over a 21-day training period, were selected for the study. Food (pelleted rat chow, Epol, Johannesburg, South Africa) and water were provided *ad libitum*. All procedures were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (Ethics No. 2004/95/5).

2.2. Surgery

Rats selected for the study (body masses 300–350 g), were anesthetized with an intramuscular (i.m.) injection of 0.4 mg/kg domitor (Novartis, SA) and 40 mg/kg ketamine hydrochloride (Anaket-V, Bayer, SA) and had a temperature-sensitive radiotransmitter (TA10TA-F40, Data Sciences, St. Paul, MN, USA) implanted intra-abdominally. Thereafter the rats were placed in a stereotaxic frame (Stoelting, IL, USA), a heating pad was placed beneath the rat to maintain core body temperature, and they were given an injection (0.1 ml) of adrenaline (10 μg) (Merck, SA) and lignocaine hydrochloride (0.02 g) (Bayer, SA) subcutaneously over an area of skull. An incision was made in the midline of the cranium to expose the skull. A 23-gauge stainless steel guide cannula (Plastics One, Roanoke, VA, USA) was placed over the right lateral cerebral ventricle. Coordinates for the guide cannula were 0.8 mm posterior to bregma, 1.5 mm lateral to the midline and 3.5 mm below the skull surface at the point of entry (Paxinos and Watson, 1998). The cannula was secured to the skull with three screws and dental cement. After surgery each rat was given a subcutaneous injection of 0.3 mg buprenorphine hydrochloride (Temgesic, Schering-Plough, SA) and ringer lactate (1.5 ml) (SABAX, Adcock, Ingram, SA) and allowed a minimum of 21 days for recovery.

2.3. Body temperature

We measured core body temperatures of rats continuously, by remote biotelemetry using temperature-sensitive radiotransmitters which had been implanted intraperitoneally (see above). Transmitter output frequency (Hz) was monitored at 5 min intervals, by a receiver plate (RTA 500, Mini-Mitter, Sunriver, OR, USA) situated beneath the cage of each animal. The frequency received by each plate was fed into a peripheral processor (DP-24 DataPort, VitalView, Mini-Mitter, Sunriver, OR, USA) connected to a personal computer and the output expressed in degrees centigrade. The telemeters were calibrated by water immersion against a high-accuracy thermometer (Quat 100, Heraeus, Germany), to an accuracy of 0.1 °C.

2.4. Voluntary wheel-running

The exercise-training wheels had a circumference of 1.06 m and each wheel was equipped with a magnet and a magnetic switch (VitalView, Mini-Mitter, Sunriver, OR, USA). Each time the wheel rotated the magnet within range of the magnetic switch, the switch closed and a turn was counted. The mechanical switches were connected to an activity input module (QA-4, VitalView, Mini-Mitter, Sunriver, OR, USA) which in turn was fed into a peripheral processor (DP-24 DataPort, VitalView, Sunriver, OR, USA) connected to a personal computer which monitored the number of wheel turns at 5 min intervals using VitalView software version 4.1 (Mini-Mitter, Bend, OR, USA).

2.5. Food intake and body mass

Food intake and body mass were measured twice daily at 08:00 (1 h after lights on) and 18:00 (1 h before lights off). Food containers were filled daily at 08:00 with 100 g of the pelleted rat chow. Food intake was quantified by subtracting the food remaining in the food container and on the cage floor from the amount of food measured at the preceding time point.