Brain, Behavior, and Immunity 40 (2014) 18–26

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Named Series: Diet, Inflammation and the Brain

Calorie restriction dose-dependently abates lipopolysaccharide-induced fever, sickness behavior, and circulating interleukin-6 while increasing corticosterone

Leah MacDonald, Agnes Hazi, Antonio G. Paolini, Stephen Kent *

School of Psychological Science, La Trobe University, Melbourne, VIC 3086, Australia

ARTICLE INFO

Article history:
Received 22 September 2013
Received in revised form 15 December 2013
Accepted 8 January 2014
Available online 15 January 2014

Keywords:
Cytokines
Food restriction
Dietary restriction
Fever
Lipopolysaccharide
Interleukin-6
Interleukin-10
Corticosterone
Sickness behavior

ABSTRACT

In mice a 50% calorie restriction (CR) for 28 days attenuates sickness behavior after lipopolysaccharide (LPS) and these mice demonstrate a central anti-inflammatory bias. This study examined the dose-dependent effect of CR on sickness behavior (fever, anorexia, cachexia) and peripheral immune markers post-LPS. Male Sprague–Dawley rats fed ad libitum or CR by 50% for 14, 21, or 28 days were injected on day 15, 22, or 29 with 50 μg/kg of LPS or saline (1 mL/500 g). Changes in body temperature (T_b), locomotor activity, body weight, and food intake were determined. A separate cohort of rats was fed ad libitum or CR by 50% for 28 days and serum levels of corticosterone (CORT), interleukin 6 (IL-6), and IL-10 were determined at 0, 2, and 4 h post-LPS. The rats CR for 28 days demonstrated the largest attenuation of sickness behavior: no fever, limited reduction in locomotor activity, no anorexia, and reduced cachexia following LPS. Rats CR for 14 and 21 days demonstrated a partial attenuation of sickness behavior. Rats CR for 14 days demonstrated a larger increase in T_b, larger reduction in locomotor activity, and larger weight loss compared to rats CR for 21 days. Serum CORT was increased at 2 h post-LPS in ad libitum and CR groups; however it was two times larger in the CR animals. Levels of IL-6 were significantly attenuated at 2 h post-LPS in the CR animals. IL-10 levels were similar post-LPS. CR results in an enhanced anti-inflammatory response in the form of increased CORT and diminished pro-inflammatory signals.

1. Introduction

It has been well established that calorie restriction (CR) can prolong lifespan (Weindruch et al., 1986), reduce the occurrence of age-related diseases such as cancer (Matsuzaki et al., 2000), lessen the severity of the neurochemical deficits and motor dysfunction seen in primate models of Parkinson's disease (Maswood et al., 2004), and attenuate the 'normal' immunosenscence seen with age (Mascarucci et al., 2002). To date there has been limited investigation on the impact CR plays on the development of fever and sickness behavior. We have previously demonstrated that a 50% CR for 28 days attenuates sickness behavior in mice (MacDonald et al., 2011). The CR mice demonstrated no increase in body temperature (T_b) normally seen after lipopolysaccharide (LPS) administration, no anorexia or cachexia, and only a limited reduction in locomotor activity.

Examination of hypothalamic mRNA post-LPS indicated an anti-inflammatory bias in the CR mice. This was demonstrated by an attenuated increase of the expression of hypothalamic cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 (mPGES-1) in CR mice at 2 h post-LPS. Hypothalamic suppressor of cytokine signaling 3 (SOCS3) mRNA expression was increased in the CR50% mice (two-fold that of the controls) at 4 h post-LPS, which led to the conclusion that SOCS3 may play an important role in the attenuation in sickness behavior seen in the CR mice (MacDonald et al., 2011). Hypothalamic mRNA levels of the anti-inflammatory cytokine interleukin-10 (IL-10) were also significantly increased in the CR mice 4 h post-LPS. SOCS3 is a known feedback regulator of cytokine production; it is induced by IL-10 (Cassatella et al., 1999), blocks IL-6 signaling (Lang et al., 2003), and aids in the anti-inflammatory actions of IL-10 (Bogdan et al., 1991).

It is important to investigate the peripheral signals that may also play a role in the CR-induced attenuation of fever and sickness behavior, as well as perhaps driving the central changes we

Abbreviations: AL, ad libitum; ANOVA, analysis of variance; CBG, corticosteroid binding globulin; CORT, corticosterone; COX-2, cyclooxygenase-2; CR, calorie restriction; CRH, corticotropin-releasing hormone; IL-6, interleukin-6; ELISA, enzyme-linked immunosorbent assays; IL-10, interleukin-10; IL-1β, interleukin-1β; LPS, lipopolysaccharide; LSD, Least Significant Difference; mPGES-1, microsomal prostaglandin E synthase-1; MANOVA, multivariate analysis of variance; NPY, neuropeptide Y; T_b, body temperature; SOCS3, suppressor of cytokine signaling 3.

* Corresponding author. Address: School of Psychological Science, La Trobe University, Bundoora, VIC 3086, Australia. Tel.: +61 3 9479 2798; fax: +61 3 9479 1956.

E-mail address: S.Kent@latrobe.edu.au (S. Kent).

0889-1591/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2014.01.005

previously observed. For example, peripheral administration of IL-10 attenuates fever induced by peripheral administration of LPS, whereas central administration of IL-10 was ineffective (Ledeboar et al., 2002). In addition to IL-10, investigations of peripheral levels of IL-6 can be seen as imperative. IL-6 is critically important for LPS-induced fever; plasma levels of LPS-induced IL-6 are highly correlated with fever (LeMay et al., 1990), and IL-6 deficient mice are unable to develop fevers post-LPS injection (Chai et al., 1996).

Increases in anti-inflammatory compounds such as corticosterone (CORT) may also play a role in attenuating sickness behavior following LPS. CORT is well established to exert anti-inflammatory effects which are achieved by firstly, increasing the transcription of anti-inflammatory cytokines and decreasing the transcription of pro-inflammatory cytokines (Smoak and Cidlowski, 2004); and secondly, by inhibiting the release of cytokines from macrophages (Barnes, 1998). CORT levels are well known to increase moderately after a period of CR (Han et al., 1995; Heiderstadt et al., 2000; Levay et al., 2010), and serum CORT concentration increases dose-dependently with increasing severity of CR, with a significant increase in serum CORT seen in rats after only 21 days at a 12.5% CR (Levay et al., 2010). A 20% CR for 20 days in hamsters increased basal levels of cortisol; however, this was not enough to alter T_b, or circulating cortisol and IL-6 compared to controls post-LPS (Conn et al., 1995). Given that the CR regimen used in this study was relatively modest (20%) and that we have previously demonstrated no increase in T_b post-LPS in mice CR to 50% (MacDonald et al., 2011) it would be interesting to see what effect a 50% CR would have on circulating CORT and IL-6 levels post-LPS.

In addition, to our knowledge a parametric analysis of how different durations of CR may impact on sickness behavior outcomes has not been conducted. It is important to establish the effect differing durations of CR may have on investigatory outcomes (in this case sickness behavior) so that the underlying mechanisms that play a role in the attenuation of sickness behavior seen in our previous studies can be further clarified.

It was shown that body-mass adjusted oxygen consumption in rats was not significantly affected by a 40% CR for two weeks or two months; however, six months CR reduced oxygen consumption by 40% (Bevilacqua et al., 2004). The same study revealed that mitochondrial production of reactive oxygen species was reduced by 53% compared to controls after two weeks, and further increased to a 57% reduction at two months, and a 74% reduction at six months, indicating a dose-dependent response to the duration of CR (Bevilacqua et al., 2004). In contrast, it was found that when comparing a short and long term (specific length not specified) 44% CR, the effect of both of these CR durations were homologous in both direction and level of change in the expression of genes that change with age (Cao et al., 2001). Furthermore, the short term CR reproduced 100% of the same effects as long term CR on urinary protein and stress response gene expression and the short term CR also reproduced 67% of the effects of long term CR on inflammatory response gene expression (Cao et al., 2001). These findings provide mixed messages as to the precise difference between short versus long term CR. It may be that depending on the variables measured that short or long term CR would be beneficial; however, not solely one or the other. The timing of the CR period needs to be optimized for specific investigations.

Previously our laboratory has demonstrated that LPS-induced sickness behavior can be attenuated by a 50% CR for 28 days in mice (MacDonald et al., 2011). The current study aims to extend that finding to another species and also to investigate possible dose-dependent responses to LPS after different durations of CR and peripheral immune related targets. It is hypothesized that a similar attenuation in sickness behavior will be seen in the rats CR to 50% for 28 days, followed by the rats CR for 21 days, and then the rats CR for 14 days will demonstrate the smallest attenuation of sickness behavior. Further, we expect that the peripheral targets investigated (CORT, IL-6 and IL-10) will further elucidate the mediating factors behind attenuated sickness behavior after CR. Given our previous findings we expect that the LPS-induced increase in CORT will be enhanced in the CR animals, the increase in IL-6 will be attenuated, and that IL-10 will be increased in the CR50% rats.

2. Methods

2.1. Animals

Ninety-five male Sprague–Dawley rats were procured from Monash SPF animal services (Clayton, Victoria, Australia) and allowed to acclimate to the facility for at least one week. During this period, standard rodent chow (Barastoc, Melbourne, Australia) and water were available ad libitum (AL). At the beginning of experimentation the rats were aged between 9 and 12 weeks old. Rats were individually housed in polypropylene basin cages ($30 \times 50 \times 15$ cm) with sawdust and tissues provided as bedding. Rats were maintained at an ambient temperature of 26 ± 1 °C, which is within the thermoneutral zone for this species (Poole and Stephenson, 1977) on a 12:12 light/dark cycle (0500–1700 h). We recently reported that AL fed rats when placed in a thermocline had a preferred ambient temperature of 23.7 ± 1.4 °C whereas CR50 rats preferred ambient temperature was 28.1 ± 0.4 °C (MacDonald et al., 2012). Animal care and experimentation was performed in accordance with protocols approved by the La Trobe University Animal Ethics Committee.

2.2. Surgery

Following acclimation, 56 of the rats were surgically implanted in the peritoneal cavity with a biotelemetry device (E-4000, Mini-mitter®, Bend, OR, USA: 23×8 mm, 1.6 g) under anesthesia as previously described (Begg et al., 2007). Rats were anaesthetized in an induction chamber using 4% isoflurane and 0.6 L/min oxygen flow and were maintained during surgery on 2.5% isoflurane and 0.4 L/min oxygen flow using a nose cone. These rats were allowed one to two weeks to recover before the initiation of the CR regimens.

2.3. Dietary regimens

Rats were divided into one of two CR regimens matched for weight, food intake, and age: AL ($n = 41$) fed ad libitum (approximately 20–30 g per day); or CR50% ($n = 54$) rats received 50% of the amount consumed by the AL animals (approximately 10–15 g per day). Thus, the dietary regimens used involved an overall reduction in food intake without modifying the constituents. The dietary composition of the AL and CR50% diets has been published elsewhere (Levay et al., 2007, 2010). The AL group met the recommended daily allowance values for all dietary and nutritional constitutes needed for growth and maintenance (Institute for Laboratory Animal Research, 1995), apart from fat. Similarly, the CR animals also met all of the recommended daily allowance values with the exception of fat and vitamin B-12. The group sizes varied across dependent variables and precise numbers used for each variable are stated in the results section. The intake of the CR groups was determined weekly based on the average daily food intake of the AL group for three consecutive days. Water was continuously provided to all groups. The dietary manipulation continued for 14, 21, or 28 days before, and four days after LPS/saline administration in Experiment 1; and for 28 days before LPS administration until