Int. J. Devl Neuroscience 48 (2016) 24–30

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Administration of branched-chain amino acids alters the balance between pro-inflammatory and anti-inflammatory cytokines

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ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form
11 November 2015
Accepted 11 November 2015
Available online 30 November 2015

Keywords:
Branched chain amino acids
Maple syrup urine disease
Brain
Cytokines

ABSTRACT

Acute leucine intoxication and neurologic deterioration can develop rapidly at any age as a result of net protein degradation precipitated by infection or psychological stress in patients with maple syrup urine disease (MSUD). Here, we investigated the effects of acute and chronic Hyper-BCAA (H-BCAA) administration on pro- and anti-inflammatory cytokines in the brains of rats. For acute administration, Wistar rats (10 and 30 days) received three injections of BCAA pool (15.8 μL/g at 1-h intervals) or saline, subcutaneously. For chronic administration, Wistar rats (7 days) received of BCAA pool or saline twice a day for 21 days, subcutaneously. Our results showed that acute administration of H-BCAA increased IL-1β (~78%; $p \leq 0.009$) and TNF-α (~155%; $p \leq 0.026$) levels in the cerebral cortex but not in the hippocampus of infant rats. Moreover, IL-6 levels were increased in the hippocampus (~135%; $p \leq 0.009$) and cerebral cortex (~417%; $p \leq 0.008$), whereas IL-10 levels were decreased only in the hippocampus (~42%; $p \leq 0.009$). However, repeated administration of H-BCAA decreased IL-1β (~59%; $p \leq 0.047$), IL-6 (~70%; $p \leq 0.009$) and IFN-γ (~70%; $p \leq 0.008$) levels in the cerebral cortex, whereas the IL-6 (~67%; $p \leq 0.009$), IL-10 (~58%; $p \leq 0.01$) and IFN-γ (~67%; $p \leq 0.009$) levels were decreased in the hippocampus. These findings suggest that a better understanding of the inflammatory response in MSUD patients may be useful to develop therapeutic strategies to modulate the hyperinflammatory/hypoinflammatory axis.

1. Introduction

Maple syrup urine disease (MSUD; OMIM 248600) is an autosomal recessive inherited metabolic disorder whose clinical presentation includes apnea, ketoacidosis, convulsion, coma, and a variable degree of mental retardation, secondary to the malfunctioning of the branched chain α-ketoacid dehydrogenase enzyme complex (BCKDC, EC 2.7.11.4) (Chuang and Shih, 2001). The BCKDC is responsible for oxidative decarboxylation of branched chain ketoacids formed by the transamination of branched chain amino acids, including leucine, isoleucine and valine (Harris et al., 1990). The metabolic defect leads to the accumulation of the branched chain amino acids (BCAAs) leucine, isoleucine, valine, and the corresponding branched-chain α-keto acids (BCKA), α-ketoisocaproic acid (KIC), α-keto-β-methylvaleric acid, and α-ketoisovaleric acid, as well as the corresponding α-hydroxy acids in tissues and body fluids (Chuang and Shih, 2001; Treacy et al., 1992).

Acute elevations of leucine and KIC cause metabolic encephalopathy and life-threatening brain edema (Strauss et al., 2006), whereas prolonged imbalances of circulating amino acids may have more subtle and long-lasting effects on brain structure and function (Kamei et al., 1992; Morton et al., 2003; Strauss et al., 2006). The mechanisms by which high concentrations of leucine and its keto acid KIC, the main metabolites accumulated in untreated patients, are toxic to the central nervous system remain

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http://dx.doi.org/10.1016/j.ijdevneu.2015.11.002
0736-5748/

poorly understood. In this context, it has been established that these metabolites compete with the transport of other critical amino acids into the brain (Araújo et al., 2001; Wajner and Vargas, 1999; Wajner et al., 2000); increase reactive oxygen species (ROS) generation and cause oxidative stress (Barschak et al., 2006; Bridi et al., 2003, 2005; Fontella et al., 2002; Mescka et al., 2011); cause DNA damage (Scaini et al., 2012a), activation of several stress kinases, mitochondrial transition pore opening (leading to Krebs cycle disruption), apoptosis and cell death in neurons and other cell types (Howell and Lee, 1963; Jouvet et al., 2000a, b; Pilla et al., 2003; Ribeiro et al., 2008; Sgaravatti et al., 2003; Zielke et al., 2002); increase acetylcholinesterase activity in the brain (Scaini et al., 2012b), and cause alterations of neurotrophin levels (Scaini et al., 2013a, b).

It is well established that acute leucine overload and neurologic deterioration can develop rapidly at any age as a result of net protein degradation precipitated by infection, surgery, injury, or psychological stress in MSUD patients (Chuang and Shih, 2001). Holecek et al. (1997) showed that skeletal muscle proteolysis and the resulting enhanced release of amino acids into the bloodstream is induced by tumour necrosis factor alpha and other cytokines. Moreover, studies have shown that excess leucine induces immunodepression, reduces ponderal growth and lymphopoiesis and depresses the production of antibodies, with the complete disappearance of serum IgG globulins (Aschkenasy, 1979; Calder, 2006; Calder and Yaqoob, 2004).

Cytokines are a group of low-molecular-weight proteins secreted by a variety of immune and non immune cells, which are classically known to play a crucial role in the stimulation or inhibition of cell proliferation, cytotoxicity/apoptosis, antiviral activity, cell growth and differentiation, inflammatory responses, and the up-regulation of surface membrane proteins (Bienvenu et al., 1998; Borish and Steinke, 2003; Kang and Der, 2004; Tayal and Kalra, 2008). Although their specific biological activities may vary, two general categories of cytokines can be distinguished: pro-inflammatory and anti-inflammatory (Meager, 2004).

The CNS has its own resident immune system in which glial cells (astrocytes and microglia) not only serve supportive and nutritive roles for neurons but also sometimes engage in several inflammatory processes that defend the CNS from pathogens and help it to recover from stress and injury (Neumann, 2001). Microglial cells exhibit great phenotypic heterogeneity and plasticity and are capable of quickly adapting to achieve an appropriate effector response to each challenge to the CNS. Recently, the concept of different states of macrophage activation was proposed, ranging from "classical" activation (pro-inflammatory or M1) to "alternative" activation (anti-inflammatory or M2), which represent the extremes of a spectrum of functional states. The classical activation state of microglia is accompanied by the induction of receptors that participate in the innate immune response (El Khoury et al., 2007), which is responsible for the pro-inflammatory milieu and has been linked to neurotoxic effects in the brain, whereas the alternative activation state is associated with the production of anti-inflammatory cytokines in the resolution phase of the inflammatory response.

The correct balance between pro- and anti-inflammatory activities is critical for preserving tissue homeostasis. Several local cues can disrupt this balance, thus promoting neuronal impairment. Emerging data show that BCAAs influence immune functions (Calder, 2006); however, the impact of BCAAs on pro- and anti-inflammatory cytokines remains unclear. Thus, in this study, we investigated the effect of acute and chronic administration of a BCAA pool (leucine, isoleucine, and valine) on TNF-α, IFN-γ, IL-1β, IL-6, and IL-10 levels in the brains of rats during their development.

2. Materials and methods

2.1. Animals

Male Wistar rats at 7, 10, or 30 days old (weighing 10–15 g, 20–25 g, and 60–80 g, respectively) were obtained from the Central Animal House of the Universidade do Extremo Sul Catarinense. The 7 and 10-day-old rats were left with their dams until the day of the experiment, and the 30-day-old rats were weaned at 21 days of life. All rats were caged in groups of 5 with free access to food and water and were maintained on a 12-h light/dark cycle (lights on at 7:00 am) at a temperature of 23 ± 1 °C. All experimental procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behaviour recommendations for animal care, with the approval of the Ethics Committee of the Universidade do Extremo Sul Catarinense (protocol number 83/2012).

2.2. Acute administration of a BCAA pool

The animals received three subcutaneous administrations of a Hyper-BCAA (H-BCAA) pool (15.8 μL/g body weight at 1-h intervals) containing 190 mmol/L leucine, 59 mmol/L isoleucine, and 69 mmol/L valine in saline solution (0.85% NaCl) or saline alone (control group). The H-BCAA pool and saline solution were given to the rats on postnatal day (PD) 10 or PD 30 ($n = 6$ per group). One hour after the last injection, the animals were sacrificed by decapitation, the brain was rapidly removed, the hippocampus, striatum, and cerebral cortex were collected for cytokine assays. The choice of the doses of the BCAAs and the age of the animals were based on a previous study by Bridi et al. (2006) showing that the administration of a BCAA pool to rats (doses and ages similar to those used in our present study) resulted in increased levels of leucine, isoleucine and valine in the blood and brain, mimicking the main biochemical finding observed in MSUD patients during crises. The representative brain areas (hippocampus, striatum and cerebral cortex) were chosen because of their general association with multiple aversive signals as well as their role in processing of memory, learning, attention, thought and motor function (Albouy et al., 2013; Bota et al., 2015; Jarrard, 1993; O'Callaghan et al., 2014; Sansom and Livesey, 2009). Moreover, previous studies from our laboratory and others have shown that BCAA cause significant changes in these areas (Bridi et al., 2003; Ribeiro et al., 2008; Rosa et al., 2015; Scaini et al., 2012a, b, 2013a, b, 2015; Sgaravatti et al., 2003).

2.3. Chronic administration of a BCAA pool

The animals were divided into the following two groups: Group I—control (saline); Group II—H-BCAA. The animals received two subcutaneous administrations of the H-BCAA pool (15.8 μL/g body weight at 12-h intervals) containing 190 mmol/L of leucine, 59 mmol/L of isoleucine, and 69 mmol/L of valine in saline solution for 21 days starting at PD 7 (last injection at PD 27; $n = 6$ per group) (Bridi et al., 2006). It is important to point out that the animals in both control and H-BCAA groups showed an appearance and behavior normal, with normal gains in weight and length over the study period. Twelve hours after the last injection, the animals were sacrificed by decapitation, the brain was quickly removed, and the hippocampus, striatum and cerebral cortex were collected for cytokine assays.

2.4. Tissue preparation

Hippocampus, striatum and cerebral cortex were homogenised (1:10, w/v) using Dounce homogenizer in ice-cold phosphate-