Available online at www.sciencedirect.com
ScienceDirect
Toxicology Letters 173 (2007) 145–150
Toxicology Letters
www.elsevier.com/locate/toxlet

Acute and chronic vitamin A supplementation at therapeutic doses induces oxidative stress in submitochondrial particles isolated from cerebral cortex and cerebellum of adult rats

Marcos Roberto de Oliveira *, José Cláudio Fonseca Moreira

Centro de Estudos em Estresse Oxidativo, Departamento de Bioquímica, Universidade Federal do Rio Grande do Sul, RS, Brazil

Received 7 May 2007; received in revised form 3 July 2007; accepted 3 July 2007
Available online 10 July 2007

Abstract

Vitamin A is an essential micronutrient to the normal brain function. However, there is an increasing concern regarding the use of vitamin A at high doses even therapeutically. Here, we show that acute and chronic vitamin A supplementation induces oxidative stress to submitochondrial particles (SMP) isolated from rat cerebral cortex and cerebellum. Both chronic and acute vitamin A supplementation at therapeutic (1000 IU/kg or 2500 IU/kg) or excessive (4500 IU/kg or 9000 IU/kg) doses induced lipid peroxidation, protein carbonylation, and oxidation of protein thiol groups in cerebral cortex and cerebellum SMP. Furthermore, vitamin A supplementation induced an increase in the superoxide ($O_2^{\bullet -}$) anion production, indicating an uncoupling in the electron transfer chain (ETC). Locomotory and exploratory activity, which are associated to cerebral cortex and cerebellum, also were affected by both acute and chronic vitamin A supplementation. Vitamin A induced a decrease in both locomotory and exploratory behavior. Together, these results show that vitamin A could be toxic at the sub cellular level, inducing mitochondrial dysfunction and altering cerebral cortex and/or cerebellum-dependent behavior.

Keywords: Vitamin A; Superoxide anion; Lipid peroxidation; Protein carbonylation; Oxidative stress; Behavior

1. Introduction

Vitamin A (also referred to as retinol) is essential to the maintenance of the central nervous system (CNS) homeostasis (Wolf, 1984). Therapeutically, it has been utilized at high doses as an interesting alternative in the treatment of people suffering from leukemia (Tsunati et al., 1990, 1991; Norum, 1993; Fenaux et al., 2001). On the other hand, there is a growing body of evidence showing that vitamin A at doses ranging from moderate to high may induce several disadvantageous effects, such as necrotizing vasculitis, fragile bone, and irritability and depression (Paydas et al., 1998; Binkley and Krueger, 2000; Myhre et al., 2003, respectively), to cite a few. As mentioned above, the therapeutic application of vitamin A at high doses may be deleterious, at least in part, to the CNS function. Indeed, there is increasing concern regarding the excessive use of vitamin A in supplemented foods among both children and adults (Lam et al., 2006). However, the mechanism by which vitamin A impairs the adult mammalian brain still remains to be elucidated.

* Corresponding author at: Departamento de Bioquímica/UFRGS, Av. Ramiro Barcelos, 2600 Anexo, Lab. 32, RS CEP: 90035-003, Brazil. Tel.: +55 51 3308 5578; fax: +55 51 3308 5540.
E-mail addresses: mrobioq@yahoo.com.br, mrobioq@gmail.com (M.R. de Oliveira).

0378-4274/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2007.07.002

We previously demonstrated that vitamin A induces oxidative stress in cultured Sertoli cells. Lipid peroxidation, protein carbonylation, and alteration on the antioxidant enzymes activities were induced by vitamin A (Moreira et al., 1997; Dal-Pizzol et al., 2000, 2001; Frota et al., 2004). Furthermore, vitamin A is genotoxic in some experimental models (Klamt et al., 2003). Moreover, mitochondria isolated from rat liver are sensitive to retinol, which induces lipid peroxidation in the mitochondrial membranes and increases superoxide ($O_2^{\bullet -}$) production, resulting in increased cytochrome *c* release from mitochondria (Klamt et al., 2005). In addiction, some metabolites of vitamin A also impair mitochondrial function (Rigobello et al., 1999).

Oxidative stress is a condition characterized by an overload in oxidants, which may culminate in cellular dysfunction (Halliwell and Gutteridge, 1999). Indeed, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved in neurodegeneration, including Parkinson's disease (PD), Alzheimer's disease (AD), and amyotrophic lateral sclerosis (ALS) (Halliwell, 2006).

The aim of this study was to investigate the consequences of acute and chronic vitamin A supplementation at therapeutic and excessive doses upon the redox state of submitochondrial particles (SMP) isolated from adult rat cerebral cortex and cerebellum, since these brain structures are known targets of vitamin A-associated toxicity (Snodgrass, 1992). Furthermore, locomotion in and exploration of an open field were analyzed because these behaviors are associated, at least in part, with cerebral cortex and cerebellum integrity. We found that vitamin A supplementation induced an increase in the production of $O_2^{\bullet -}$, an index of uncoupling in the electron transport chain (ETC). Additionally, vitamin A supplementation induced lipid peroxidation, protein carbonylation, and decreased the protein thiol content in SMP isolated from rat cerebral cortex and cerebellum. Locomotion and exploration were decreased by vitamin A supplementation acutely or chronically.

2. Experimental procedures

2.1. Animals

Adult male Wistar rats (280–320 g) were obtained from our own breeding colony. They were caged in groups of five with free access to food and water and were maintained on a 12-h light–dark cycle (7:00–19:00 h), at a temperature-controlled colony room (23 ± 1 °C). These conditions were maintained constant throughout the experiments. All experimental procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication number 80-23 revised 1996) and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care. The Ethical Committee for animal experimentation of the Federal University of Rio Grande do Sul approved our research protocol.

2.2. Drugs and reagents

Arovit® (retinol palmitate), a water-soluble form of vitamin A, was purchased from Roche, São Paulo, SP, BR. All other chemicals were purchased from Sigma, St. Louis, MO, USA. The preparation of the vitamin A treatments occurred by protecting it from light, since either vitamin A or its derivatives are photosensitive.

2.3. Experimental model

The animals were treated once a day during three different periods: acutely (3 days or 7 days), or chronically (28 days). All treatments were carried out during night period (*i.e.* when the animals are more active and take a greater amount of food) in order to ensure maximum vitamin A absorption, since this vitamin is better absorbed during or after a meal. Animals were gently gavaged daily with vehicle (0.15 M NaCl), 1000 IU/kg, 2500 IU/kg, 4500 IU/kg, or 9000 IU/kg in a maximum volume of 0.8 mL during each period of interest. Adequate measures were taken to minimize pain or discomfort.

2.4. Behavioral task

The behavioral task occurred 15 h after the last treatment, and was performed between 14:00 and 16:00 h. Before each session, the animals were allowed to adapt to the experimental room for at least 30 min. The open field task was carried out in 60 cm × 40 cm open field surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 12 equal rectangles by black lines. The animals were placed on the same initial rectangle and were left to freely explore the arena for 5 min. The number of crossings of the black lines and rearings were counted over this time. In behavioral analysis, rats were used only once.

2.5. Oxidative stress in submitochondrial particles

The rats were sacrificed by decapitation 24 h after the last vitamin A administration. Briefly, to obtain submitochondrial particles (SMP), cerebral cortex and cerebellum were dissected, homogenized in 230 mM mannitol, 70 mM sucrose, 10 mM Tris–HCl and 1 mM EDTA (pH 7.4). Freezing and thawing (three times) the mitochondrial solution gave rise to superoxide dismutase-free SMP. The SMP solution was also washed (twice) with 140 mM KCl, 20 mM Tris–HCl (pH 7.4) to ensure Mn-SOD release from mitochondria. To quantify superoxide ($O_2^{\bullet -}$) production, SMP was incubated in reaction medium consisted of 230 mM mannitol, 70 mM sucrose, 10 mM HEPES-KOH (pH 7.4), 4.2 mM succinate, 0.5 mM