available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Intragastric administration of evodiamine suppresses NPY and AgRP gene expression in the hypothalamus and decreases food intake in rats

Jinghong Shi, Jianqun Yan, Qi Lei, Junjie Zhao, Ke Chen, Dejun Yang, Xiaolin Zhao, Yuan Zhang*

Department of Physiology and Pathophysiology, Key Laboratory of Environment and Genes Related to Diseases, Ministry of Education, Xi'an Jiaotong University School of Medicine, Xi'an, Shaanxi 710061, China

ARTICLE INFO

Article history:
Accepted 24 September 2008
Available online 15 October 2008

Keywords:
Evodiamine
Leptin
NPY
AgRP
Food intake
Body weight

ABSTRACT

Evodiamine (Evo), an alkaloidal component extracted from the fruit of Evodiae fructus (*Evodia rutaecarpa* Bentham, Rutaceae), decreases the body weight of rats through a poorly defined mechanism. The hypothalamus is one of the areas in the brain linked to the control of food intake and energy expenditure. We postulate that Evo mediates this activity by modulating feeding-related peptides in the hypothalamus. We investigated the effects of Evo on food intake, body weight, the mRNA expression and peptide level of feeding-related peptides in the hypothalamus, in male rats. The juvenile rats of 5 weeks old were used at the start of the experiment. Evo (40 mg/kg or 4 mg/kg) was administered intragastrically for 25 days, and food intake and body weight of rats were recorded daily. Blood samples were collected for leptin radioimmunoassay (RIA). Real-Time PCR was used to analyze the mRNA expression. Western Blotting and immunohistochemistry were used to analyze the peptide. Our results show that intragastric administration of Evo (40 mg/kg) decreased rate of food intake and body weight increase following rat growth, reduced orexigenic neuropeptide Y (NPY) and agouti-gene related protein (AgRP) mRNA levels and NPY peptide level in the arcuate nucleus (ARC) of the hypothalamus, but it increases the circulating level of leptin. Intragastric administration of a smaller dose of Evo (4 mg/kg) was ineffective. These data suggest that Evo decreases food intake, and therefore body weight, partly by down-regulating NPY and AgRP mRNA expression and peptide expression in the ARC of the hypothalamus.

* *Corresponding author*. Fax: +86 29 82655199.
E-mail address: jqyan@mail.xjtu.edu.cn (J. Yan).

Abbreviations: Evo, Evodiamine; NPY, neuropeptide Y; AgRP, agouti-gene related protein; MCH, melanin concentrating hormone; POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; α-MSH, α-melanocyte-stimulating hormone; MC4R, melanocortin receptor-4; ARC, the arcuate nucleus; LHA, lateral hypothalamus area; PVN, paraventricular nucleus; VMH, ventromedial hypothalamic nucleus; DMH, dorsomedial hypothalamic nucleus

0006-8993/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2008.09.091

1. Introduction

Evodiamine (Evo) is a major alkaloidal component of the dried, unripe fruit of *Evodia rutaecarpa* Bentham (Rutaceae), and as an agonist of vanilloid receptor1 (VR1) has no perceivable taste. Previous studies have indicated that Evo has a number of pharmacological actions such as decreasing body weight, regulating body temperature, anti-tumor activity, pain relief, heart protection, decreasing blood pressure and inducing endocrine effects (Kobayashi et al., 2001; Wang et al., 2008; Takada et al., 2005; Chiou et al., 1992; Huang et al., 2005; Kan et al., 2007). It has been proposed that Evo exhibits capsaicin-like anti-obesity actions through stimulation of VR1 in the primary sensory neurons (Kobayashi et al., 2001). Oral Evo significantly decreased the body weight, perirenal fat weight and epididymal fat weight of rats on a high-fat diet (Kobayashi et al., 2001). Recently, studies have shown that dietary supplementation with Evo could ameliorate diet-induced obesity in mice and inhibit adipogenesis (Wang et al., 2008). There is evidence suggesting that Evo inhibits adipogenesis by stimulating ERK/MAPK signaling which down-regulates the expression of adipocyte transcription factors and insulin-induced Akt signaling. Evo clearly shows anti-obesity effects in UCP1-deficient mice, and may trigger a UCP1-independent mechanism to prevent diet-induced obesity. Evo also improves leptin resistance and insulin sensitivity in mice (Wang et al., 2008). However, the mechanism involved in the process remains unclear and needs further investigation.

The hypothalamus and its neural circuits play an important role in regulating feeding behavior (Clifford et al., 2002; Williams et al., 2001). Numerous neuropeptides produced by the neurons of the arcuate nucleus (ARC) in the hypothalamus are involved in regulating food intake and body weight (Clifford et al., 2002; Williams et al., 2001). It has been demonstrated that these neuropeptides have different actions. Leptin inhibits food intake and increase energy expenditure through inhibition of orexigenic neuropeptide Y (NPY)/agouti-gene related protein (AgRP) neurons and stimulation of anorexigenic pro-opiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART) neurons in the ARC (Korner et al., 2001; Baskin et al., 1999; Kristensen et al., 1998; Cowley et al., 2001). The α-melanocyte-stimulating hormone (α-MSH), through binding with high affinity to melanocortin receptor-3 (MC3R) and MC4R, inhibits food intake (Cone et al., 1999). AgRP, a MC4R antagonist, binds with MC4R to compete with α-MSH to enhance the effect of NPY (Ollmann et al., 1997). Melanin concentrating hormone (MCH) is mainly expressed in the lateral hypothalamus area (LHA), and the central administration of MCH stimulates feeding (Rossi et al., 1997). Orexigenic peptides interact with anorexigenic peptides to balance energy metabolism.

The aim of our study was to determine whether Evo has effects on hypothalamic neuropeptides and serum leptin levels, and to examine whether the mRNA expression of NPY, AgRP, MCH, POMC, CART, and MC4R in the rat hypothalamus changed following administration of Evo. The information provided by this study may help to explain the molecular mechanism of the central mechanisms of Evo anti-obesity action.

2. Results

2.1. The effect of Evo on food intake and body weight

The group treated with Evo 40 mg/kg had a significant decrease in food intake from the 12th day onwards as compared with the control group ($P<0.05$). The group treated with Evo 4 mg/kg had no significant difference compared with the control group ($P>0.05$), as shown in Fig. 1A. At the 12th treatment day, food intake of the Evo 40 mg/kg group (21.98±0.52 g/d) and control (23.86±0.56 g/d) was significantly different (one-way ANOVA, $P=0.021$). At the 21st day, the Evo 40 mg/kg group (24.44±0.53 g/d) and control (27.20±0.65 g/d) was significantly different (one-way ANOVA, $P=0.001$). The mean food intake in the Evo 40 mg/kg group at 5 time points from 12th to 24th day were 90%, 86%, 86%, 88%, and 87% of the control group, respectively.

The group treated with Evo 40 mg/kg had a noticeable fall in rate of body weight increase from the 15th to 25th days as compared with the control group ($P<0.05$). The group treated

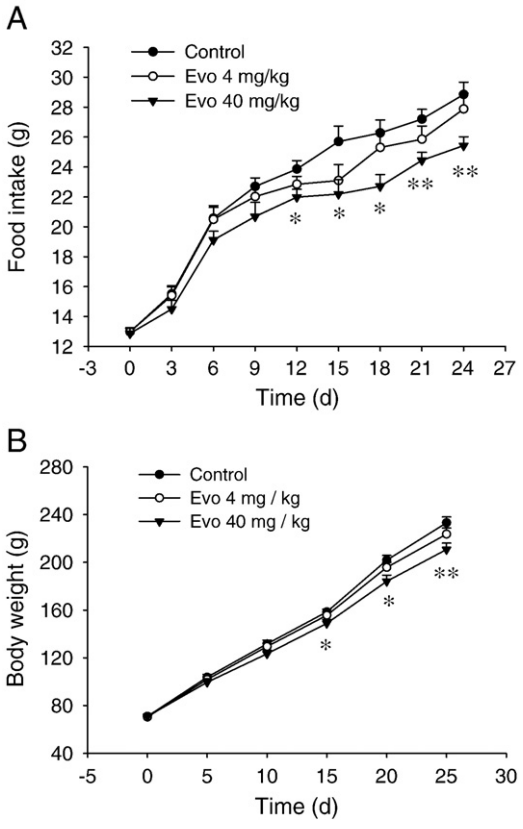

Fig. 1 – The effects of Evo on food intake (A) and body weight (B). The group treated with Evo 40 mg/kg had a significant decrease in food intake and a noticeable fall in body weight from the 12th or 15th to 25th days as compared with the control group. *$P<0.05$ and **$P<0.01$, compared with the control group ($n=15$).