Available online at www.sciencedirect.com

SCIENCE DIRECT®

Life Sciences 77 (2005) 2804–2813

Life Sciences

www.elsevier.com/locate/lifescie

Developmental changes of the expression of the genes regulated by retinoic acid in the small intestine of rats

Yuko Ogura[a], Kazuhito Suruga[a], Sachiko Takase[b], Toshinao Goda[a,*]

[a]*Laboratory of Nutritional Physiology and COE Program in the 21st Century, University of Shizuoka School of Food and Nutritional Sciences, 52-1 Yada, Shizuoka-shi 422-8526, Japan*

[b]*Department of Nutrition and Health Sciences, Siebold University of Nagasaki, Nagasaki 851-2195, Japan*

Received 9 December 2004; accepted 3 May 2005

Abstract

Retinoic acid (RA) serves as a hormone-like nutrient and it plays pivotal roles in cellular differentiation and proliferation in various tissues including the small intestine. In this study, we aimed to explore a possible role of RA signaling in the developing rat small intestine of perinatal (embryonic and newborn) and suckling–weaning transition period, and we investigated the changes in the expression of several genes regulated by RA. Northern blot analysis showed that both retinal dehydrogenase 1 (RALDH1) and retinal dehydrogenase 2 (RALDH2) mRNA levels were higher in 19-day fetal (2 days before birth) small intestine and then declined after birth. Retinoid X receptor alpha (RXRα) mRNA and retinoic acid receptor alpha (RARα) mRNA levels in the small intestine showed high levels in perinatal period compared with suckling–weaning transition period. RA-target genes such as retinoic acid receptor beta (RARβ) and cellular retinol-binding protein, type II (CRBP II) mRNA levels were significantly increased in the perinatal small intestine. Furthermore, mRNA levels of hepatocyte nuclear factor-4 (HNF-4), which is one of the possible RA-target gene and a transcription factor regulating CRBP II gene expression, was also increased in the perinatal small intestine. These results suggest that the possible perinatal RA production by RALDHs might regulate various RA-target genes including CRBP II and RARα through RXRα or HNF-4 in the small intestine.

Keywords: Retinoic acid; RALDH; Nuclear receptor; CRBP II; Small intestine; Gene expression

* Corresponding author. Tel.: +81 54 264 5533; fax: +81 54 264 5565.
E-mail address: gouda@fns1.u-shizuoka-ken.ac.jp (T. Goda).

0024-3205/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2005.05.028

Introduction

Retinoic acid (RA), an active metabolite of vitamin A (retinol), plays an important role in the development and differentiation of various organs through modulations of the transcription of various genes (Balmer and Blomhoff, 2002). These actions of RA are mediated by two classes of the nuclear hormone receptor superfamily, the RA receptors (RARs) and the retinoid X receptors (RXRs) (Ross et al., 2000). RARs are activated by not only all-trans RA but also 9-cis retinoic acid as ligand, whereas RXRs bind exclusively 9-cis retinoic acid as ligand. RA is enzymatically synthesized from retinol by two sequential oxidation steps, which include the oxidation of retinol to retinal and the oxidation of retinal to RA (Napoli, 1999). It has been reported that an irreversible oxidation of retinal to RA is catalyzed by cytosolic retinal dehydrogenases, which are members of the aldehyde dehydrogenase (ALDH) family and designated as RALDHs (Napoli, 1999).

Small intestine is the first gateway for contact and uptake of various kinds of environmental factors such as many kinds of nutrients, drugs and xenobiotics. Marked changes in the small intestinal functions occur especially in the perinatal and suckling–weaning transition periods. It is well known that nutrient factors and several hormones such as glucocorticoid and thyroid hormone play important roles in the regulation of the small intestinal gene expressions throughout the suckling –weaning transition period (Henning, 1981; Thomson and Keelan, 1986). On the other hand, during the perinatal stage the small intestine undergoes the initial morphological and functional maturation, which is accompanied by the abrupt induction of various genes related to nutrient uptake and metabolism (Henning, 1981; Thomson and Keelan, 1986). However, precise mechanisms of perinatal induction of these gene expressions are still unknown. RA also plays pivotal roles during entire life stage in growth, maintenance and differentiation of various epithelial cells including those in the small intestine (Henning, 1985; McCormack et al., 1996; Plateroti et al., 1993). Therefore we hypothesized that RA might be one of the regulatory factor of these genes in the perinatal small intestine. In the present study, we investigated the changes in the transcript levels of RA-synthesizing enzymes RALDHs, retinoid receptors and their putative target genes such as cellular retinol-binding protein, type II (CRBP II and hepatocyte nuclear factors (HNFs) in the perinatal small intestine.

Materials and methods

Animals

Sprague–Dawley rats were obtained from Japan SLC (Hamamatsu, Japan). Pups were kept with their mothers, and both mother and pups were given free access to water and a standard laboratory chow diet (MF, Oriental Yeast, Tokyo, Japan) throughout the experimental period. All rats were housed under a light cycle with 12-h darkness from 19:00 to 7:00. The embryos were dissected at the age of 19-day fetus (2 days before birth). Newborn rat pups were killed at the age of 0, 1, 3, 5, 13, 17, 20, and 27 days old pups by decapitation between 10:00 and 12:00. The experimental procedures used in the present study met the guidelines of the animal usage committee of the University of Shizuoka.

RNA analysis

The small intestine was washed with ice-cold 0.9% NaCl solution. A portion (100 mg) of the small intestine was used for total RNA extraction. Total RNA was isolated using TRIzol reagent (Invitrogen,