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Retinal dehydrogenase gene expression in stomach and small intestine of rats during postnatal development and in vitamin A deficiency

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Abstract Retinal dehydrogenase (RALDH) catalyzes the oxidation of retinal to all-*trans* and 9-*cis* retinoic acid, which function as ligands controlling RAR and RXR nuclear receptor-signaling pathways. We have recently shown the expression of RALDH transcript in the stomach and small intestine by reverse transcription polymerase chain reaction [Bhat, P.V., Labrecque J., Dumas, F., Lacroix, A. and Yoshida, A. (1995) Gene 166, 303–306]. We have examined RALDH expression in the stomach and small intestine before and during postnatal development and in vitamin A deficiency by assaying for mRNA levels and protein as well as for enzyme activity. In −2 day fetuses, RALDH expression was high in the small intestine, whereas RALDH protein was not detectable in the stomach. However, expression of RALDH was seen in the stomach after birth, and gradually increased with age and reached the highest level at postnatal day 42. In the intestine, RALDH expression decreased postnatally. Vitamin A deficiency up-regulated RALDH expression in the stomach and small intestine, and administration of retinoids down-regulated the RALDH expression in these tissues. These results show the differential expression of RALDH in the stomach and small intestine during postnatal development, and that vitamin A status regulates the expression of RALDH gene in these tissues.

Key words: Retinoic acid; Retinal dehydrogenase; Gene expression; Vitamin A deficiency

1. Introduction

Retinoic acid (RA), an active metabolite of vitamin A, is essential for growth, development, and maintenance of normal cellular differentiation [1–3]. RA regulates gene transcription acting through ligand-dependent transcription factors: the RA receptors (RARs) and the retinoid X receptors (RXRs) [4,5]. The pathway of RA synthesis involves first the oxidation of retinol to retinal and then the oxidation of retinal to RA [6,7]. We previously reported an aldehyde dehydrogenase designated retinal dehydrogenase (RALDH) from rat kidney that was able to oxidize retinal to all-*trans* and 9-*cis* RA [8,9]. Recently, we identified RALDH transcripts in the stomach and small intestine of rats by reverse transcription polymerase chain reaction [10]. We have also shown that the expression of RALDH mRNA is primarily in the epithelial lining of these tissues by in situ hybridization [11].

Vitamin A is essential for the maintenance of the epithelial structure of the stomach and small intestine [12]. In vitamin A deficiency, the forestomach shows hyperkeratosis and the number of goblet cells of the small intestine severely declines [13]. Because RA is involved in the epithelial cell differentiation of the stomach and small intestine, and also because the endogenous RA levels in embryonic tissues are controlled by developmentally regulated mechanisms that are likely to include modulation of expression and/or activity of enzymes catalyzing RA synthesis [14,15], it was of interest to examine the developmental pattern of RALDH gene expression in these epithelial tissues. Further, we also studied the effects of vitamin A status on RALDH gene expression in these tissues.

2. Materials and methods

2.1. Materials

Vitamin A deficient diet containing AIN-76 salt mixture [16] was obtained from ICN Nutritional Biochemicals, Cleveland, OH. Retinoids leupeptin, pepstatin and phenylmethylsulfonyl fluoride (PMSF) were purchased from Sigma, St. Louis, MO. TRI pure isolation reagent was obtained from Boehringer Mannheim. Quickhyb reagent, random priming kit for Northern analysis were purchased from Stratagene. Enhanced chemiluminescence (ECL) reagents were from Pierce.

2.2. Animals

Sprague-Dawley rats were obtained from Canadian Breeding Farm, Quebec. Fetuses were delivered by cesarian section on day 19 of pregnancy (−2 days). For Northern blot, Western blot and enzyme assay analysis, the organs from fetuses ($n = 40$) or young animals ($n = 80$) were pooled (4–65 individual organs), snap frozen in liquid nitrogen and stored at −80°C before analysis. Tissues were from 19 day embryo (E19) and postnatal rats on day 0, 2, 6, 12, 16, and 42 (p0–p42).

Vitamin A deficiency was induced in specially prepared weanling male rats as described earlier [17]. When the rats reached a plateau in growth rate, they were divided into two groups. The deficient group (A−) was maintained on the diet without supplementation; the repleted groups (RA+, ROL+) were supplemented orally with all-*trans* RA (100 μg/day) and retinol (1.5 mg/day) in 0.1 ml corn oil for 1 week and 4 days, respectively. Rats from each group were killed, stomach and small intestine were quickly removed, and the contents were washed in cold phosphate-buffered saline (PBS) and frozen in liquid nitrogen and stored at −80°C till analyzed.

2.3. Northern analysis

Total tissue RNAs were isolated with the TRI pure extraction reagent according to the manufacturer's protocol. 15 μg of total RNA was separated in 1.1% agarose gels containing 1.2% formaldehyde and 1×MOPS running buffer. RNA was transferred to nytran membranes by capillary blotting using 10×SSC and cross-linked to the membrane by UV irradiation. The blots were prehybridized and hybridized at 68°C using Quickhyb reagent. Blots were washed twice with 2×SSC, 0.1% SDS at room temperature for 15 min and then washed with 0.1×SSC, 0.1% SDS at 65°C for 30 min. Actin (2.0 kb, Oncor, Gaithersburg, MD) was used as a control probe. All cDNA probes were labeled with [^{32}P]dCTP using Prime-It random prime kit.

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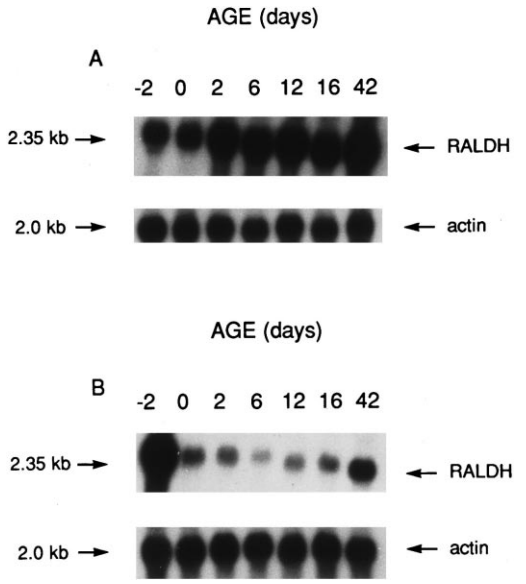

Fig. 1. Expression of RALDH mRNA in stomach (A) and small intestine (B) of rats during development. 15 μg of total RNA was separated on 1.1% agarose gel as described in Section 2. Actin (2.0 kb) was used to ensure loading of similar amounts of RNA.

2.4. Western blot analysis

Tissues were homogenized in 1.0 ml of 100 mM ice-cold Tris buffer (pH 8.0) containing 3 mM EDTA, 1 μg/ml leupeptin and pepstatin, 0.5 mM PMSF. The homogenates were centrifuged at $10\,000\times g$ for 10 min and the supernatants were collected. 20 μg of total protein (determined by the method of Bradford) [18] was boiled in sample buffer, separated by 8% polyacrylamide sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to Hybond nitrocellulose (Amersham). Blots were blocked with 0.05% Tween-20 in 5% BSA before incubation with antibodies. Immunoreactive protein was detected using an ECL Western blotting system. The antibody raised against phenobarbital-induced aldehyde dehydrogenase (kindly provided by Dr. Ronald Lindhal), which cross-reacts with RALDH, was used in the Western blot analysis.

2.5. Enzyme assay

The $100\,000\times g$ supernatant fractions from tissues were used to assay RALDH activity. In a standard assay, 1.0 mg of supernatant protein was incubated at pH 7.2 with 141 μM all-*trans* retinal in the presence of 1.61 mM DTT, 603 μM NAD, and 0.02% Tween-80 at 37°C for 1 h in a final volume of 250 μl. The enzymatic product RA formed was quantified by high pressure liquid chromatography as described earlier [19].

3. Results and discussion

Level of RALDH transcripts in stomach and small intestine during development is shown in Fig. 1. RALDH mRNAs are detectable in stomach during late intrauterine development, and reach highest levels postnatally between days 2 and 42 (p20–p42). In small intestine, the highest levels of RALDH transcripts are observed before birth (day 19, −2 day), and then the levels decline progressively thereafter. Changes in mRNA levels were followed by corresponding changes in protein levels (Fig. 2) and RALDH activity (Fig. 3). Variations in the levels of RALDH protein expression and activity paralleled the expression of RALDH mRNA (Figs. 1–3) in both tissues; although a lag in protein expression compared to mRNA expression was seen at −2 days and 42 days in stomach and small intestine respectively (Figs. 1 and 2). This could be due either to the low sensitivity of the antibody to detect the low concentration of RALDH protein or to the high stability of mature primary RNA transcripts.

Vitamin A deficiency caused an increase in the levels of RALDH mRNA and protein in stomach and small intestine (Figs. 4 and 5). Supplementation of either RA or retinol to vitamin A deficient rats decreased RALDH mRNA and protein levels, demonstrating that vitamin A levels modulate the expression of RALDH gene in these tissues.

Several studies have shown that RA has a well characterized role in the development and maintenance of epithelial cells throughout life [1,12]. The expression of RALDH gene in several types of epithelia including stomach and small intestine [11] indicates an important role of this enzyme in RA synthesis needed for cell differentiation and maturation. During development of the stomach, a single layer of gastric epithelium becomes stratified and then changes from stratified to simple columnar epithelium by day 17.5 of gestation. Just before birth, mucus cells and parietal cells are formed. As the rat develops, the funding mucosa shows remarkable changes. Immediately after birth, the mucous epithelium differentiates into short, premature fundic glands. The fundic glands rapidly elongate from 1 day to 2 weeks after birth. The various types of cells in fundic gland differentiate and mature in accordance with development of the animal [20,21]. Thus, the process of differentiation and maturation of the cells of stomach epithelium occurs during postnatal development of the rat. The observation of an increase in the RALDH expression during postnatal development of the stomach (Figs. 1–3) suggests a role for this enzyme in producing RA required for normal differentiation and maturation. Just before birth, intestinal mucosa of the rat displays a high level of structural development characterized by villi lined with a single layer of columnar epithelial cells that have well defined microvilli at their absorptive surface [22]. This structural development of the small intestine coincides with high expression of RALDH gene, indicating crucial requirement of

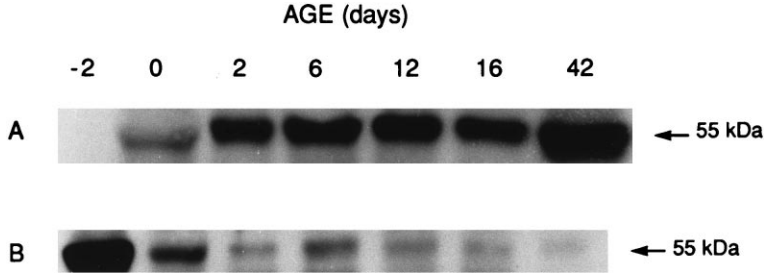

Fig. 2. Western analysis of RALDH protein. Expression of RALDH protein in stomach (A) and small intestine (B) of rats during development. 20 μg of total protein was separated on 8.% SDS-PAGE and RALDH protein was detected by immunoblot as described in Section 2.

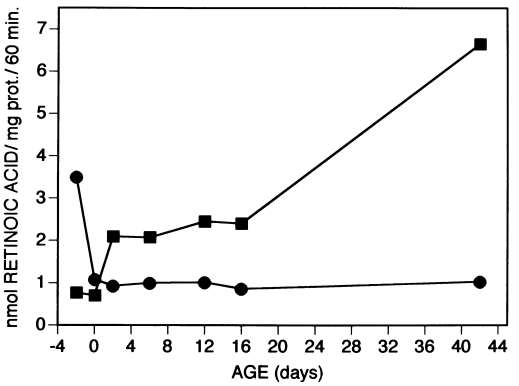

Fig. 3. Activity of RALDH for retinal oxidation in stomach (■) and small intestine (●) of rats during development.

RA for these processes. However, it is not clear why the expression of RALDH declines after birth in the intestine, although there is continuous removal and proliferation of intestinal epithelium occurs during postnatal development [23]. Studies have shown that RA is present in the small intestine of 16.5 dpc embryos and adults [24,25]. Since intestine is one of the main organs involved in retinoid uptake, storage, turnover, and excretion [26], it is possible that adult small intestine may take up RA from the circulation or absorb it from the diet and RA present in the tissue may account for this form of RA and not in situ generated RA. It is also possible that a high level of RA in the adult small intestine taken up from the circulation or absorbed from the diet may down-regulate the expression of RALDH, as we have shown that RA levels modulate the expression of RALDH gene (Figs. 4 and 5). At present, the mechanism(s) by which RA down-regulates RALDH gene in stomach and small intestine is not know. However, it is tempting to speculate that there may be a RA response element in the promoter of RALDH gene that may interact with one of the nuclear RAR receptors and regulate RALDH gene expression.

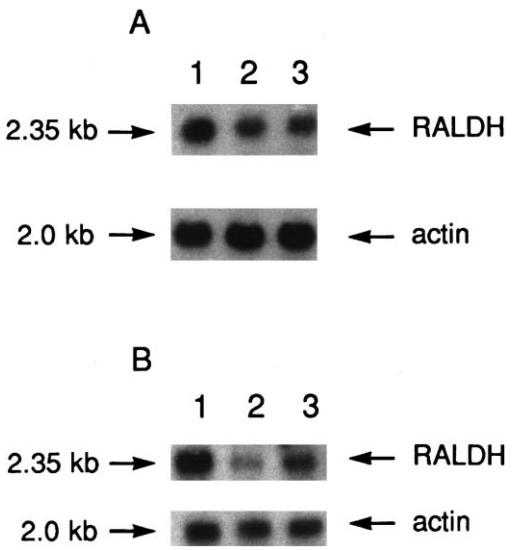

Fig. 4. Comparison of RALDH transcripts in stomach (A), and small intestine (B) from A−, RA+, and ROL+ rats. 15 μg of total RNA was separated on 1% agarose gels and hybridized to RALDH cDNA followed by actin cDNA. Lanes 1, 2, 3 are RNAs from A−, RA+ and ROL+ stomach and small intestine, respectively.

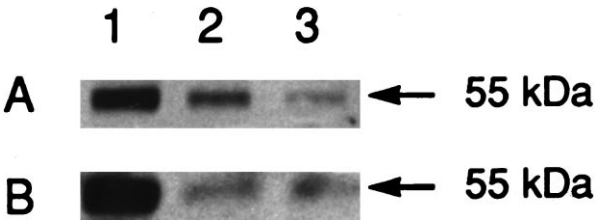

Fig. 5. Expression of RALDH protein in stomach (A) and small intestine (B) from A−, RA+, and ROL+ rats. Lanes 1, 2 and 3 are proteins from A−, RA+, and ROL+ stomach and small intestine, respectively.

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