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The possible roles of homeobox protein, Cdx-2 for the expression of LPH gene during postnatal development

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Abstract

The expression of intestinal lactase-phlorizin hydrolase (LPH) gene normally decreases after completion of weaning in almost all mammals. To elucidate the mechanism whereby LPH gene expression is regulated during the suckling–weaning period, we studied the effects of the thyroid hormone (T_3) on LPH gene expression in the small intestine during postnatal development in the rat. Firstly, we measured LPH mRNA level in rat jejunum at 5, 13, 20 and 27 days after birth. The amount of LPH mRNA at 27 days was significantly lower than that at 5 days. The transcript level of Cdx-2, which is a putative transcriptional factor for regulation of LPH gene expression, was also significantly decreased after 21 days. The binding of nuclear protein to the *cis* element CE-LPH1 on the promoter region of the LPH gene was reduced at the end of the weaning period. Daily intraperitoneal (i.p.) injection of T_3 for 6 days during days 22–27 significantly reduced LPH mRNA level by day 27 (50%, $P<0.01$), but injection of T_3 during days 8–13 did not. Moreover, i.p. T_3 injection during days 22–27 was accompanied by a reduction in the level of Cdx-2 mRNA. Our study suggests that the decrease in the LPH gene expression during the weaning period is associated with a reduction of Cdx-2 expression caused by thyroid hormone.

Keywords: LPH; Thyroid hormone; Postnatal development; Cdx-2

Introduction

Lactase-phlorizin hydrolase (LPH) is a brush border β-glycosidase which hydrolyzes lactose, the principal sugar found in milk. Lactase activity and LPH gene expression are high during the suckling period, but their levels decrease during weaning (Buller et al., 1990). Recently, several groups have demonstrated that LPH gene expression is positively regulated by Cdx-2, which is one of the homeobox proteins. Cdx-2 binds to the *cis*-element CE-LPH1 on the LPH gene promoter (Spodsberg et al., 1999). Cdx-2 is highly expressed in the small intestine and colon, and is also detected in the pancreas (James and Kazenwadel, 1991; Silberg et al., 2000; Suh et al., 1994). It is known that Cdx-2 is important for the development and maintenance of intestinal epithelium, as well as that of LPH gene expression (Chawengsaksophak et al., 1997; Suh and Traber, 1996; Tanaka et al., 1997). A recent study reported that deficiency in one of the thyroid hormone receptor (TR) subtypes, TRα, in mice led to reduction of the expression of both Cdx-2 and LPH genes in the postnatal small intestine (Plateroti et al., 1999). This suggested that thyroid hormone should be important for transcription of both LPH and Cdx-2 in the postnatal small intestine. By contrast, previous studies have demonstrated that serum levels of thyroid hormones, e. g., L-tri-iodothyronine (T_3) and L-tetra-iodothyronine (thyroxine, T_4) are elevated during weaning (Koldovsky, 1985), when lactase activity is declined. Several studies have demonstrated that i.p. injection of thyroid hormone to rats during weaning reduces LPH gene expression in the small intestine (Celano et al., 1977; Yeh and Moog, 1974). Therefore, it is very likely that response of LPH gene expression to thyroid hormone should be varied depending on the period during the postnatal development.

In this study, we examined whether LPH and Cdx-2 genes are coordinately regulated by thyroid hormone during the suckling–weaning period, and whether the binding of Cdx-2 to

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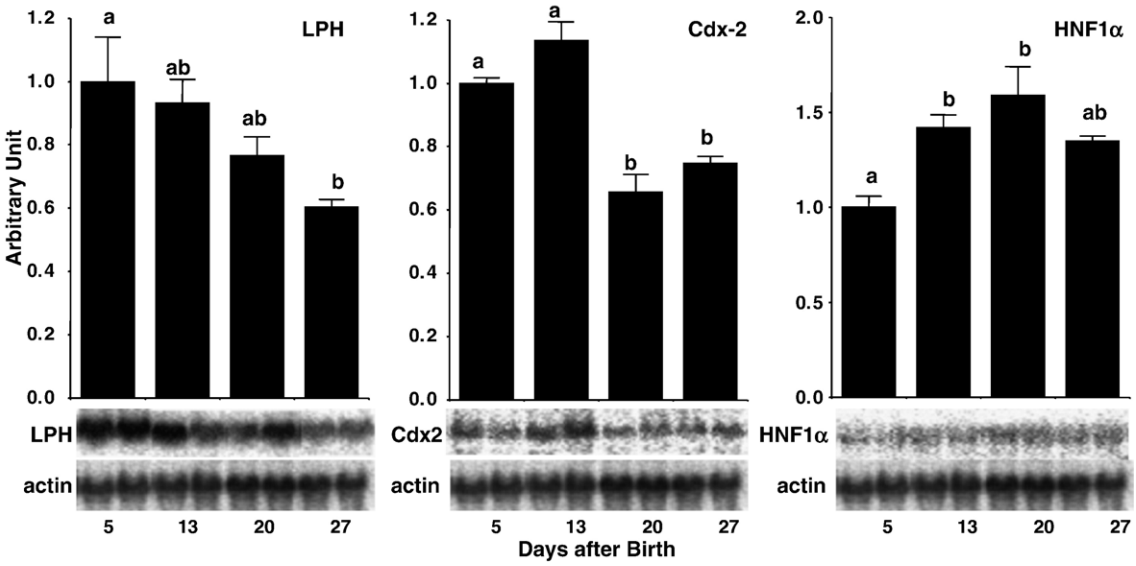

Fig. 1. Developmental changes of LPH, Cdx-2 and HNF-1α mRNA levels in rat small intestine. LPH, Cdx-2, and HNF-1α mRNA levels were quantified by Northern blot hybridization. The mRNA levels are represented in the graphs as relative signal density after normalization with β-actin mRNA level as an internal control. Values represent means±SEM, $n=3–4$. Values not sharing a common superscript are significantly different from one another by Tukey's multiple range test.

the *cis*-regulatory element CE-LPH1 on the LPH gene decreases after weaning.

Materials and methods

Animals

Sprague–Dawley suckling rats (Japan SLC, Hamamatsu, Japan) were kept with their mothers, and both mothers and pups were given free access to a standard laboratory chow diet (MF, Oriental Yeast, Tokyo, Japan) throughout the experimental period. The rat pups were killed by decapitation between 15:00 and 16:00 h at the age of 5, 13, 20 and 27 days. In another experiment, rat pups were intraperitoneally injected with 0.3 μg/g body weight T_3 at 9:00 h daily for 6 consecutive days prior to killing at 13:00 at 13 and 27 days of age. Control animals were injected with 1 mM NaOH (vehicle) only. The experimental procedure used in the present study met the guidelines of the Animal Usage Committee of the University of Shizuoka.

RNA analysis

The entire small intestine was flushed with ice-cold 0.9% NaCl solution. The jejunoileum extending from the ligament of Treitz to the ileocecal valve was divided into two equal parts along its length. A portion (100 mg) was excised from the middle of the proximal half of the jejunoileum (jejunum) and immediately used for RNA extraction. Total RNA was extracted by the acidified guanidinium thiocyanate method as described by Chomczynski and Sacchi (Chawengsaksophak et al., 1997; Chomczynski and Sacchi, 1987). Northern blot analysis was performed using ^{32}P-labeled cDNA probes as described previously (Goda et al., 1995; Yasutake et al., 1995). The cDNA probes used for Northern blot are described for rat LPH (Goda et al., 1995), rat β-actin (Goda et al., 1995), rat hepatocyte nuclear factor (HNF)1α, a fragment corresponding to +1 to +1887 (Chouard et al., 1990), and rat Cdx-2, a fragment corresponding to +1 to +933 (Unigene ID; Rn.64495). The specific mRNA signals from Northern blots were quantified using an image analyzer (BAS 2000, Fuji Film, Tokyo, Japan) using ^{32}P-labeled cDNA probes, and the signals were standardized for β-actin mRNA signal.

Electrophoretic mobility shift assays

Intestinal nuclei were isolated from each jejunum as described previously (Suruga et al., 1999). The nuclear proteins

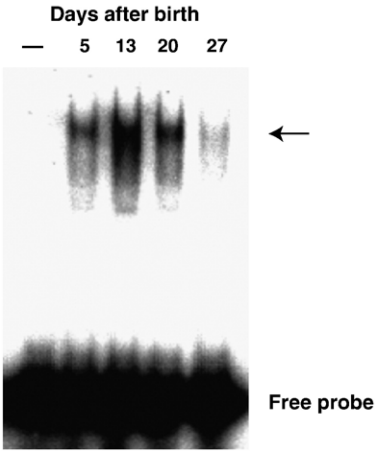

Fig. 2. EMSA of CE-LPH1 using nuclear proteins extracted from the small intestine of suckling and weaning rat. Nuclear proteins were extracted from the rat's small intestine at 5, 13, 20 and 27 days of age. The nuclear proteins (1 μg) were incubated with ^{32}P-radiolabeled CE-LPH1 probe prior to polyacrylamide gel electrophoresis.