Volume 248, number 1,2, 39–42 FEB 07096 May 1989

Discrepancy between the intestinal lactase enzymatic activity and mRNA accumulation in sucklings and adults

Effect of starvation and thyroxine treatment

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Received 23 January 1989; revised version received 13 March 1989

The accumulation profile of intestinal lactase mRNA was investigated in suckling and adult rats and pigs. We found no correlation between the lactase enzymatic activity and the accumulation of the messenger at both developmental stages. Modulation of lactase activity by starvation or thyroxine treatment had no effect on lactase mRNA accumulation in the rat intestine. These results confirm that thyroxine modulates lactase expression essentially at the post-transcriptional level.

Lactase; Phlorizin hydrolase; mRNA accumulation; Enzyme activity; Development; Thyroxine; (Rat, Pig)

1. INTRODUCTION

Lactase-phlorizin hydrolase (LPH), found in most suckling mammals, is a major intrinsic component of the intestinal brush border membrane, which is involved in the hydrolysis of lactose and glycosylceramides. The mature 130–180 kDa enzymatic complex is processed intracellularly from a high molecular mass precursor [1–4] and incorporated into the plasma membrane probably as a dimer [5]. Recently, the cloning and sequencing of the adult human and rabbit LPH cDNA led to a better understanding of the structure of the enzyme and to some proposals concerning the processing of the precursor [6].

The developmental pattern of intestinal lactase activity is now well-documented in mammals [7–9]. Lactase activity, which is high during the suckling period, drops at weaning, reaching adult values that are about 10-times lower than in the neonates. Simultaneously, the rate of biosynthesis of the brush border lactase complex decreases at weaning [10]. From experiments carried out in rat, we have demonstrated that this pattern is under hormonal control [11–13]. Indeed, changes in the enzymatic activity and in the amount of lactase protein, as well as in its processing and glycosylation follow endogenous changes of concentration of thyroxine that accompany weaning. Moreover, physiological treatments which alter the amount of thyroxine (injection of exogenous thyroxine, starvation, thyroidectomy) also act on lactase activity as well as on the molecular form of the enzymatic complex.

In order to identify the level of regulation of lactase expression, we have determined the accumulation profile of LPH mRNA under different physiological conditions and correlated these data to the pattern of lactase activity.

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2. MATERIALS AND METHODS

Intestinal epithelial cells were scraped from porcine and rat proximal jejunum and used for RNA preparation [14] and determination of the specific activity of lactase in purified brush border membranes [11,15]. Polyadenylated RNA were loaded onto 0.8% agarose/17% formaldehyde gels, run in Mops buffer, and transferred onto nitrocellulose filters.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

Oligonucleotides were synthesized chemically [16] and labelled using polynucleotide kinase [17]. Hybridization between labelled oligonucleotides and immobilized RNA was performed at 22°C in a solution containing 5 × SSC, 5% formamide, 0.02% polyvinylpyrrolidone, 0.02% ficoll, 0.1% SDS, and 100 μg/ml of denatured salmon sperm DNA. Filters were washed under the stringency conditions described in the text and autoradiographed.

3. RESULTS

Rabbit as well as human LPH cDNAs contain tandemly organized boxes presenting a high degree of sequence similarity among themselves [6]. For detection of pig and rat LPH mRNAs we therefore synthesized chemically a 20-base oligonucleotide probe derived from the rabbit LPH cDNA sequence, in a region which is well conserved among the internally similar boxes. This synthetic oligonucleotide, of sequence 5′-GCCTGGGGCA-GGTCCCAGTG-3′, was termed MR24.

We first conducted experiments in order to define standard conditions of hybridization between the oligonucleotide probe MR24 and pig and rat LPH mRNAs. For this purpose, we extracted polyadenylated RNA from the jejunal mucosa of a 3-week-old suckling pig, a 6-month-old adult pig, 13-day-old suckling rats and 3-month-old adult rats. The RNAs were separated onto agarose/formaldehyde gels, blotted onto nitrocellulose filters and hybridized with MR24. The filters carrying out the rat and pig RNA were washed at 25 and 37°C, respectively, in a solution containing 0.5 × SSC and 0.1% SDS. Control experiments were performed on these RNAs using as probe the synthetic oligonucleotide designated MR61, which is derived from the human villin cDNA sequence [18]. As reported in [19], this oligonucleotide hybridized in rat and pig intestine to the 3.9 kb villin messenger which accumulated in greater amount in adults than sucklings (fig.1, lanes 5–8).

As illustrated in fig.1 (lanes 1–4), the oligonucleotide probe originating from the rabbit LPH cDNA sequence hybridized in the jejunal mucosa to a single polyadenylated RNA of about 6100 nucleotides in pig and 6300 nucleotides in rat. No hybridization signal was detected in porcine liver and kidney (not shown). These RNAs were similar in size to the rabbit and human LPH messengers [6] and we therefore assumed that they correspond to rat and pig LPH mRNA. In rats but not in pigs, faint background hybridization was occasionally detected at the level of the contaminating ribosomal RNA. Surprisingly, approximately the same amount of LPH messenger was found in suckling and adult animals, although in the latter lactase activity dropped after weaning. The present results are in accordance with those obtained in rabbit, which indicate that lactase activity does not follow the accumulation profile of the lactase messenger during development (Sebastio et al., submitted).

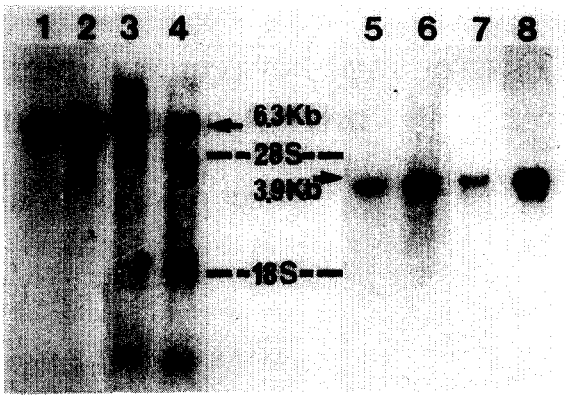

Fig.1. Hybridization between oligonucleotides MR24 and MF61, and pig and rat intestinal RNAs. About 2 μg polyadenylated RNA, extracted from jejunum of suckling and adult pigs (lanes 1,2, respectively) and rats (lanes 3,4, respectively), were separated by electrophoresis at 100 V for 4 h, transferred onto nitrocellulose filters, and hybridized with probe MR24 which detects the LPH messenger. The same RNAs were also hybridized with probe MF61, which detects the villin messenger (lanes 5–8). Migration of ribosomal RNA is indicated.

Since the amount of circulating thyroxine modulates the lactase activity, we then analyzed LPH mRNA accumulation under different physiological conditions. Polyadenylated RNA was extracted from the jejunal mucosa of 13-day-old suckling rats, 13-day-old rats previously injected daily with 1 μg thyroxine (Roche) per g body wt over 4 days, control 13-day-old rats previously injected daily with 0.9% NaCl for 4 days, 4-month-old fed rats and 4-month-old rats starved for 2 days. Mucosal samples were taken from the same animals in order to determine the specific activity of lactase (table 1). As expected and as has been well documented [11–13], the high activity found in sucklings, either untreated or NaCl-injected, dropped in adults. An exogenous increase

Table 1

Jejunal lactase specific activity in rat under various physiological conditions

	Animals				
	Sucklings			Adults	
	Untreated (n = 10)	Thyroxine-injected (n = 10)	NaCl-injected (n = 10)	Starved (n = 6)	Fed (n = 6)
Lactase activity (mU/mg protein)	349[a] ± 33	178[b] ± 15	328[a] ± 31	99[c] ± 8.8	48[d] ± 5.1

The specific activity of lactase was determined from jejunal brush border membranes [11]. Results are presented as means ± SE. The number of animals is given in parentheses. $p < 0.01$, as determined using Wilcoxon's test for unpaired samples: a ≠ b ≠ c ≠ d

in circulating thyroxine promoted a precocious decline in lactase activity in sucklings. Starvation for 48 h, which is known to depress the level of thyroxine, caused an approx. 2-fold increase in lactase activity in adults. These enzymatic variations followed changes in the amount of anti-mature-lactase immunoreactive material found in the cells [12].

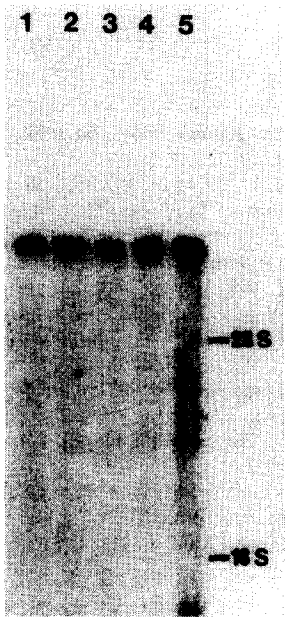

Fig.2. Accumulation profile of rat LPH RNA under different physiological conditions. Polyadenylated RNAs extracted from the jejunum of untreated sucklings (lane 1), sucklings injected with thyroxine (lane 2), sucklings injected with NaCl (lane 3), starved adults (lane 4) and fed adults (lane 5), were separated by electrophoresis at 90 V for 14 h and transferred onto a nitrocellulose filter. LPH messenger was detected using probe MR24. Each lane contained about 2 μg RNA except lane 5, which contained about 4 μg RNA. Migration of ribosomal RNA is indicated.

The various intestinal rat RNAs were separated by 14-h electrophoresis in agarose/formaldehyde gels at 90 V and blotted onto nitrocellulose filters. The filters were first hybridized with the synthetic oligonucleotide which detects the villin messenger, which showed that thyroxine treatment as well as starvation did not modify the normal profile of accumulation of this mRNA during development (not shown).

The hybridization profile with the LPH probe, MR24, is depicted in fig.2. Two conclusions were drawn from a comparison of the results obtained at the RNA and enzymatic levels. (i) Independently of the physiological conditions and the actual lactase activity, no significant difference was observed in the steady-state amount of LPH mRNA. (ii) Even under electrophoresis conditions designed to display small variations in size, the size of the LPH mRNA was identical in the various animals that we analyzed.

4. DISCUSSION

Our observations lead to some speculation as to the regulation of lactase expression. As already mentioned, a good correlation has been established between the amount of circulating thyroxine and the enzymatic activity of lactase and the number of

lactase molecules. The present data suggest in addition that the pattern of lactase activity is not related to the accumulation profile of LPH mRNA. Although we cannot exclude the possibility that the constant accumulation of LPH messenger during development masks a differential rate of biosynthesis and degradation of the mRNA, this work suggests, as proposed previously [13], that the major step in regulation of lactase expression might not occur at the genetic level, but rather at the translational and/or post-translational level(s). Furthermore, the present results corroborate a previous report indicating that lactase stimulation by starvation in adult rats is essentially actinomycin D-insensitive [20]. In consequence, enterocytes should contain a pool of LPH mRNA and/or of lactase protein or precursor, while regulatory factors such as thyroxine would modulate the amount giving rise to the active brush border enzymatic complex.

Could there be a physiological basis for the presence of high levels of LPH mRNA throughout life, despite the low lactase activity found in adults? The assignment of a function in adults to the LPH precursor or its amino-terminal half might help in answering this puzzle.

In conclusion, our results strongly suggest that LPH expression is regulated essentially at the post-transcriptional level. Further analysis is then needed to investigate in detail the biosynthesis and processing of the high molecular mass polypeptide precursor, in order to determine precisely the molecular mechanisms involved in the regulation of lactase expression.

Acknowledgements: This work was supported by INRA grant 88/4667. We thank A. Staub (INSERM U.184) for synthesis of the oligonucleotides and F. Gossé for excellent technical assistance.

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