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CORTISONE AND THYROXINE MODULATE INTESTINAL LACTASE AND SUCRASE mRNA LEVELS AND ACTIVITIES IN THE SUCKLING RAT

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Glucocorticoids and thyroxine modulate postnatal intestinal sucrase and lactase activities. Whether changes in enzyme activity are accompanied by changes in enzyme mRNA levels were determined in day 6 rats given thyroxine, cortisone, or thyroxine plus cortisone and killed 3 day later. Cortisone induced precocious expression of jejunal sucrase activity which was enhanced when cortisone plus thyroxine was administered; sucrase mRNA changed in parallel. Jejunal lactase activity was unaffected by thyroxine and was increased after cortisone, but not after thyroxine plus cortisone. Jejunal lactase mRNA levels increased equally after cortisone or after cortisone plus thyroxine. Thus, cortisone induces coordinated increases in sucrase and lactase activities and in corresponding mRNA levels. Thyroxine only enhances cortisone induced sucrase expression and antagonizes cortisone by depressing lactase activity post-translationally.

Intestinal lactase (L) and sucrase-isomaltase (SI) are brush border membrane integral enzymes, the former essential for the digestion of lactose and the latter for sucrose. In the rat, intestinal L activity initially is expressed prenatally and is maintained postnatally at high levels until late in the third week after birth when animals are weaning (1-3). Conversely, sucrase (S) activity appears and surges at a time while lactase activity decreases (2-5). The developmental changes in these two enzymes are closely related to optimal intestinal digestion of maternal milk or of carbohydrates in the adult diet. These concomitant changes serve as important intestinal maturation markers and their regulation has been extensively studied. Both

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Abbreviations used: Cortisone=C; Thyroxine=T_4; Sucrase-isomaltase=SI; Lactase=L.

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the pituitary-adrenal and pituitary-thyroidal axis modulate these enzyme changes, since hypophysectomy, adrenalectomy or thyroidectomy impairs these developmental changes unless corticosteroids and/or thyroid hormones are provided (6-8).

The detailed mechanism by which corticosteroids and thyroid hormones modulate postnatal changes in S and L activities remain incompletely understood. The appearance and increase in intestinal S activity appear to parallel SI mRNA levels during normal development and after cortisone administration (9). Postnatal decrease in L activity has been reported either to parallel (10) or not to parallel (11-12) changes in L mRNA levels. Recently we reported that thyroxine (T_4) and cortisone (C) cooperate in the induction of SI synthesis and S activity (13). Although these hormones also stimulate L synthesis, they promote a fall in intestinal L content and activity (13). Whether T_4+C induced enzymic changes are accompanied by changes in intestinal SI and L mRNA levels is unknown.

In this report, we show that T_4 cooperates with C in increasing intestinal SI mRNA levels and that T_4 does not reduce C stimulated L mRNA levels. Coordinated increases in intestinal SI protein content, sucrase activity and SI mRNA levels after T_4 plus C suggest that the mRNA level is rate limiting in SI expression. The observation that C increases intestinal L mRNA levels is the first to show that L mRNA levels can be upregulated postnatally. C plus T_4 do not suppress L mRNA levels, but significantly reduce intestinal L content and activity suggesting that T_4 reduces intestinal L activity post-transcriptionally.

MATERIALS AND METHODS

Animals and experimental treatments

Sprague-Dawley strain rat pups were used as previously described (13). Day 6 rats were separated into 4 groups and were subcutaneously injected with a single dose of vehicle (control), thyroxine (T_4, 1 μg/g body weight), cortisone (C, 50 μg/g) and T_4+C respectively. Day 6 rats were used, since T_4 and C cooperatively stimulate intestinal S and L synthesis at that age (13). Animals were killed on day 9 by decapitation and the intestine was quickly rinsed with ice cold aseptic saline and blot dried on a paper towel. The jejunum (the mid-third of small intestine) was weighed and immediately homogenized with a Tissue-mizer (Tekmar, Cincinnati, OH) in 4 N guanidinium thiocyanate in 10 mM Tris-HCl buffer, pH 7.0, containing 1% mercaptoethanol. Total RNA was isolated as described by MacDonald et al (14). A 3 cm segment of intestine immediately above the jejunum was stored at -72° C until enzyme assay.

Measurements of jejunal L and S activities and contents

Jejunal L and S activities were determined by the method of Dalqvist (15) and protein was determined by the method of Lowry et. al (16). Jejunal L and SI protein contents were determined by rocket immunoelectrophoresis (13).

Analysis of jejunal L and SI mRNA levels

The recovery of jejunal total RNA was measured by absorbance at 260 nm wavelength using a Hitachi Spectrophotometer (Danbury, CT). Isolated total

RNA from different animal groups showed similar OD 260/280 ratios in the range of 1.9-2.2. For L mRNA measurement, slot blot and Northern analysis were performed using ^{32}[P]-labeled cDNA probes (a 1.8kb L cDNA segment) as described previously (11). To normalize the amount of RNA sample applied to each lane, ^{32}[P]-labeled β-actin 40 mer (Oncogene Science Inc. Manhasset, NY) was included in the hybridization solution. For SI mRNA determination, dot and Northern blot analyses were performed using Nonradioactive Nucleic Acid Detection Kit (Boehringer Mannheim, Indianapolis, IN). The SI cDNA probe, a 827 b segment, was prepared from p-Gem PRSI-1, a kind gift from Dr. Traber (17). For Northern blot, total RNA (10 μg) from the various animal groups was fractionated by 1% agarose-formaldehyde gel electrophoresis, gels were stained with ethidium bromide to localize 28S and 18S bands and RNA was transferred to Nytran membranes (ICN Biomedicals, Costa Mesa, CA) by passive diffusion in 20X SSC (3M NaCl, and 0.3M sodium citrate, pH 7.0) solution overnight. For slot or dot blot, total RNA was denatured in 7.4% formaldehyde in 15X SSC and applied to Nytran membranes. The membranes were air dried, baked at 80^{o} C for 2h before hybridization. Radioactivity of ^{32}[P]-L cDNA and β-actin probe was adjusted to 1x10^{6} cpm/ml and the digoxigenin-SI cDNA was about 40 ng/ml in the hybridization solution. After hybridization, membranes were washed once in 2X SSC, 0.1% SDS at room temperature for 20 min, once in 0.1X SSC, 0.1% SDS at 42^{o}C for 10 min and 2 changes of 0.1X SSC, 0.1% SDS for 10 min each at room temperature. To detect ^{32}[P]-probes, the wet membranes were wrapped with Saran Wrap (Dow Brands Inc, IN) and used to make contact exposure of an X-Omat AR film (Eastman Kodak Co, Rochester, NY) for 24-48 h at room temperature. The autoradiograms were scanned with a Zeineth Soft Laser Scanning Densitometer (Biomed Instrument, Fullerton, CA) to determine the lactase mRNA and β-actin levels in the same lane. L mRNA levels were normalized to that of β-actin. To detect the digoxigenin-SI cDNA probes, immunochemical procedure recommended by Boehringer Mannheim (Indianapolis, IN) was used.

RESULTS

Effect of T_4 and/or C on jejunal S and L activity and contents

Jejunal S activity was undetectable or negligible in day 9 control rats or rats given T_4 , but was induced to high levels after C and was further induced after T_4+C (Figure 1). Jejunal SI protein content increased in parallel with induced S activity after C and after T_4+C, being 1.57$\pm$0.20 and 2.50$\pm$0.21 ng/mg mucosal protein respectively. Jejunal L activity was high in day 9 controls, did not change after T_4 administration, and increased significantly after C (Figure 1, $P<0.01$). This increase was abolished when C was administered together with T_4 (Figure 1). Jejunal L protein contents and L activity also changed in proportion (data not shown).

Effect of T_4 and/or C on jejunal SI and L mRNA levels

Dot and Northern blot analyses demonstrated that SI mRNA appeared after C and this induction was further enhanced after T_4+C (Figure 2). Northern analysis showed that the size of SI mRNA was about 6.5 kb. The 28S and 18S rRNA levels after ethidium bromide staining showed some differences in RNA applied, but this did not correspond to induced SI mRNA (Figure 2).

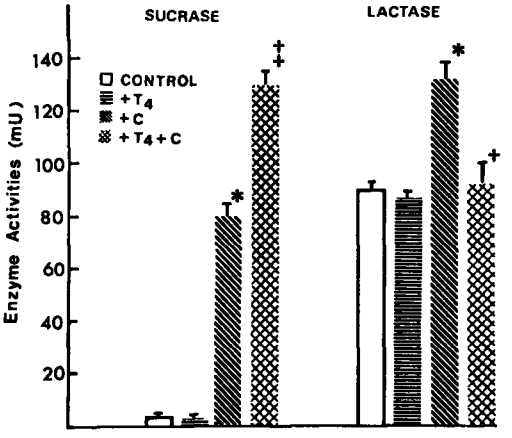

Figure 1. Effect of thyroxine and cortisone on jejunal S and L activities. Rat pups at 6-day-old were either given vehicle (control, 8 rats), thyroxine (T_4, 1 μg/g, 7 rats), cortisone acetate (C, 50 μg/g, 8 rats) or T_4+C (8 rats) and were killed 3 day later. S and L specific activity are expressed as mU (nmole substrate hydrolyzed/mg protein/min). Data are means ±SE. Analyzed by unpaired student's t-test: *P < 0.001 vs. control; †P < 0.01 and ‡P < 0.001 vs. +C.

Slot and Northern blot analyses showed that jejunal L mRNA levels were unchanged after T_4, increased after cortisone and remained high after T_4+C (Figure 3). The high level of jejunal L mRNA after T_4+C contrasted with the

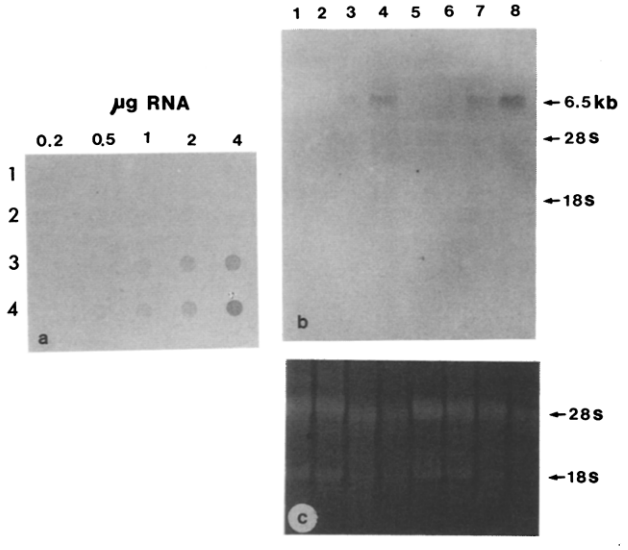

Figure 2. Dot blot and Northern blot analyses of jejunal SI mRNA expression. For dot blot (a), total RNA (10 μg) from control, T_4-, C-, and T_4+C-treated rats was denatured by formaldehyde and applied to a Nytran membrane in row 1, 2, 3 and 4 respectively. For Northern analysis (b), total jejunal RNA (10 μg/lane) from control (lane 1, 5), T_4- (lane 2 and 6), C- (lane 3 and 7) or T_4+C-treated rats were fractionated by agarose-formaldehyde gel electrophoresis, transferred to a Nytran membrane. RNA blots were hybridized to Digoxigenin-SI cDNA and visualized by immunochemical staining techniques. RNA in gel stained with ethidium bromide before transfer to the membrane showed 28S and 18S rRNA in each lane (c).

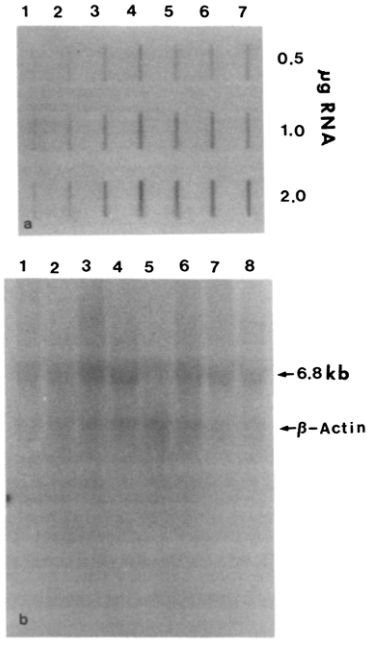

Figure 3. Slot blot and Northern analyses of jejunal L mRNA levels after hormone treatments as in the Figure 2. For slot blot (a), control, T_4-, C- and T_4+C-treated rats are in row 1-2, 3, 4-5, and 6-7 respectively. For Northern blot analysis (b), control, T_4, C and T_4+C rats are in lane 1 and 5, 2 and 6, 3 and 7, and 4 and 8 respectively. RNA blots were hybridized to ^{32}[P]-L cDNA and β-actin probes. Arrows indicate the location of 28S and 18S rRNA in the Northern blot.

decrease in L activity (Figure 1). Changes in jejunal L mRNA levels normalized to β-actin mRNA showed an increase after C (Figure 4, $P<0.05$ vs. control) and this increase was maintained after T_4+C ($P<0.01$ vs. control).

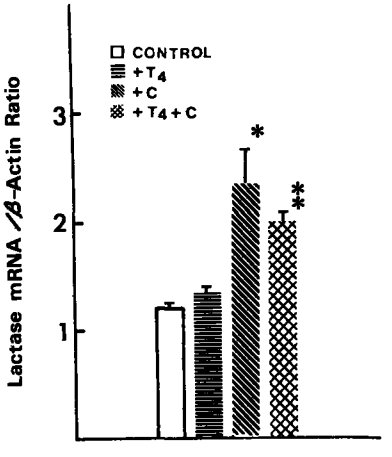

Figure 4. Effect of T_4 and/or C on jejunal L and β-actin mRNA ratios. Autoradiograms from Northern blot were scanned with a laser microdensitometer and the ratio of peak absorbance areas between L mRNA and β-actin mRNA in the same lane were calculated. Values are means ± SE from 4 rats in each group. *$P< 0.05$ and **$P <0.001$ vs. control or T_4-treated rats.

DISCUSSION

Changes in intestinal S activity that occur during normal development or by precocious glucocorticoid induction have been reported to parallel changes in SI mRNA content in rabbits and in humans (18,19) and in rats using rabbit SI cDNA probes (9). The present study used a rat SI cDNA probe and showed that T_4 enhances the effect of C by elevating SI mRNA levels proportionally to SI protein contents. This is consistent with our observations that T_4+C stimulates greater SI protein production than C alone (13). Thus, steady-state SI mRNA levels appear to determine intestinal SI synthesis, SI accumulation and S activity. We previously reported that T_4 alone did not induce SI expression until day 13 and that T_4 induced SI showed a lower S activity than SI induced by C or by T_4+C (13). Whether post-translational, as well as pretranslational, regulatory mechanisms mediate T_4 induction of SI expression in rats older than day 13 remains to be clarified.

The decrease in intestinal L activity that occurs late in the third week after birth has been reported either to be associated with (10) or not associated with decreased L mRNA levels (11,12). T_4 has been reported to precociously decrease jejunal L activity, but not L mRNA levels (20). The present study shows a parallel increase in L activity and L mRNA following C administration. However, after T_4+C, L mRNA levels remain high while jejunal L content and activity fall. We previously reported that C or T_4+C increases jejunal L production (13). Thus, C increases: (a) steady-state level of L mRNA, (b) jejunal L synthetic rate, and (c) jejunal L content and activity. The effects of C on (a) and (b) are unaltered after T_4+C, but L activity and content is reduced probably due to increased enzyme turnover (21).

The present data show that T_4 and glucocorticoids alter SI expression by regulating SI mRNA levels and alter L expression through changing both L mRNA levels and by post-translational degradation. The precise mechanism by which T_4 and C increase steady-state SI and L mRNA levels is unknown, but these hormones might act directly to stimulate SI or L gene expression, or indirectly through other proteins or factors. Recently, the 5'-flanking region of human L gene up to 1026 nucleotides has been reported to contain no consensus sequences for glucocorticoid receptor binding (22), suggesting that glucocorticoids either stabilize L mRNA or indirectly stimulate L gene transcription. The mechanism by which T_4+C increases post-translational degradation of L also remains incompletely known. Studies are in progress to answer the last question.

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