0031-3998/92/3203-0306$03.00/0
PEDIATRIC RESEARCH

Vol. 32, No. 3, 1992
Printed in U.S.A.

Synergistic Effects of Thyroxine and Dexamethasone on Enzyme Ontogeny in Rat Small Intestine

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ABSTRACT. The synergistic effects of dexamethasone (DEX) and thyroxine (T_4) on the postnatal maturation of the 13-d-old rodent small intestine has been studied. Previous studies have shown that hydrocortisone and T_4 produced a synergistic response in enzyme maturation. However, T_4 elevates corticosteroid-binding globulin, which reduces the clearance of hydrocortisone. Thus, the apparent synergy between T_4 and hydrocortisone may have been due to increased glucocorticoid availability. DEX, which does not bind to corticosteroid-binding globulin, was given (d 8–12) at 25 pmol (*i.e.* 0.01 μg)/g body wt/d as established by a dose-response study in which this dose of DEX induced one third the maximum response in sucrase activity. In this way, synergy with T_4 (130 pmol/g body wt/d, *i.e.* 0.1 μg/g body wt/d, d 5–12) could still be observed. Glucoamylase, lactase, acid β-galactosidase, alkaline phosphatase, and sucrase activities were determined in two regions of the small intestine. Overall, the results for the two hormones administered alone showed intestinal maturation to be not significantly affected in the T_4 group and partially stimulated in the DEX group. When combined, DEX + T_4 synergistically increased jejunal sucrase, ileal glucoamylase, and duodenal alkaline phosphatase, and lowered ileal acid β-galactosidase. The striking exceptions to the general pattern were two brush border enzymes that normally decline during intestinal maturation, namely ileal alkaline phosphatase and jejunal and ileal lactase. For these enzymes, DEX alone did not elicit precocious maturation, and there was no evidence for a synergistic interaction of these two hormones. Serum corticosterone concentrations also were measured. When corticosterone concentrations were compared with enzyme activity, no correlation was found. Thus, DEX + T_4 act synergistically in the maturation of four enzymes in the rodent small intestine. Lactase and ileal alkaline phosphatase are regulated differently. Further studies are required to determine the mechanism through which T_4 enhances the DEX effect. (*Pediatr Res* 32: 306–311, 1992)

Abbreviations

BW, body weight
CBG, corticosteroid-binding globulin
DEX, dexamethasone
GC, glucocorticoid
T_4, thyroxine

Postnatal maturation of the rodent small intestine is characterized by significant modification in enzyme status during the 3rd week of life. Enzymes such as sucrase and glucoamylase have almost undetectable activity at birth and increase rapidly in activity around postnatal d 16, achieving adult levels between d 21 and 24. Lactase and acid β-galactosidase have high activities at birth that decline during the 3rd postnatal week. These changes in enzyme status coincide with weaning and represent important adaptations to the nutrient differences between milk and solid food (1, 2).

Studies regarding enzyme development have suggested that modifications in enzyme activity are influenced by the hormonal milieu of the rat pup (1, 2). Corticosterone, which increases in concentration toward the end of the 2nd postnatal week just before the time of weaning (3), is an important factor in intestinal maturation, because GC have been shown to directly affect enzyme development *in vitro* (4, 5) and to induce precocious maturation of enzyme activity *in vivo* (1, 6–8). In contrast, T_4, which increases in concentration early in the 2nd postnatal week (9) and which has been studied extensively with regard to small intestinal enzyme ontogeny (1, 2), has not been shown to affect the intestinal epithelium directly (10). Assessment of the role of T_4 in intestinal ontogeny is complicated by the fact that alterations of thyroid status have been shown to influence circulating concentrations of both endogenous and exogenous GC (11, 12). Thus, for example, when the same doses of exogenous corticosterone were administered to hypothyroid and hyperthyroid rat pups, the circulating concentration of corticosterone was almost 3-fold greater in the hyperthyroid pups (12). This influence of thyroid status on circulating concentrations of GC has been shown to be due to T_4 causing increased production of CBG (11, 13, 14). By increasing the circulating concentration of CBG (11) and the percent bound fraction of corticosterone (12, 15), T_4 decreases the volume of distribution and the metabolic clearance rate of corticosterone (15). This results in an increased concentration of circulating corticosterone (12, 14).

Although studies in adrenalectomized animals have made a convincing case that T_4 in the absence of GC does not influence intestinal development (16), the fact remains that under normal circumstances both hormones are present. Thus, the possibility that T_4 enhances the effects of GC is highly plausible. Previous studies showing synergism between the two hormones have been compromised by the interactions noted above. Specifically, all such studies have used hydrocortisone or cortisone as the exogenous GC. Synergism has been deduced from the observation that combined administration of these GC and T_4 has greater effects on intestinal enzymes than does the GC alone (8, 17, 18). However, in view of the recent findings regarding T_4 and CBG (described above), these effects actually may have been simply a reflection of higher circulating hydrocortisone in the animals receiving hydrocortisone plus T_4. To reexamine the question of synergy between T_4 and GC, we performed a study using the synthetic steroid DEX, which does not bind to CBG and thus

Received December 3, 1991; accepted April 20, 1992.
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Supported by Grant HD 14094 from the National Institutes of Health.

yields circulating concentrations unaffected by CBG status (14) and by thyroid status.

Because previous studies indicated that conclusions regarding intestinal development cannot be based on the behavior of a single enzyme (19, 20), our study encompassed five small intestinal enzymes. The enzymes chosen for study included two enzymes that normally increase in activity during postnatal development, sucrase and glucoamylase (1, 6, 21), and two enzymes that show declining activity during postnatal development, lactase and acid β-galactosidase (1, 7). Each of these four enzymes was assayed in both jejunum and ileum because of recent evidence indicating that enzyme activity is controlled differently in these regions (19, 22). The 5th enzyme studied was alkaline phosphatase, whose activity rises with development in the proximal small intestine and declines in the distal small intestine (20). It was therefore assayed in both the duodenum and the ileum.

MATERIALS AND METHODS

Chemicals. [1,2,6,7-^{3}H]-corticosterone was purchased from DuPont-NEN (Boston, MA). Dextran (molecular weight 500 000), L-thyroxine (T_4), DEX, lactose, glycogen, glucose oxidase reagent [glucose (Trinder) 100], *o*-nitrophenyl-β-D-galactopyranoside (ONPG), *p*-nitrophenyl phosphate (PNP), *p*-nitrophenol, charcoal, and BSA were obtained from Sigma Chemical Co. (St. Louis, MO). Glucose (Trinder) 100 contains glucose oxidase together with peroxidase and a quinoneimine dye, which allows stoichiometric production of a colored compound from the reducing equivalents of the glucose oxidase reaction. Dextran-coated charcoal suspension was prepared by adding 37.5 mg/mL activated charcoal to 20 μM (*i.e.* 10 mg/mL) dextran in 40 mM sodium phosphate, pH 7.9. Ultrapure grade sucrose from Schwartz/Mann (Orangeburg, NJ) was used for sucrase assay. DEX for injection was prepared in 550 mM ethanol in 150 mM NaCl. T_4 was prepared in 150 mM NaCl containing 0.3 mM NaOH.

Animals. Timed-pregnant Sprague-Dawley rats from Charles River Breeding Laboratories (Portage, MI) were received on the 14th day of gestation. They were housed individually in opaque polystyrene cages and provided with food (Rodent Lab Chow #5001, Ralston Purina Co., St. Louis, MO) and water *ad libitum*. Animal quarters were air conditioned (21 ± 1°C) and maintained on a 12-h light/dark schedule with lights on at 0600 h. On the due date, cages were checked every 3 h for deliveries, and the birth date was regarded as d 0. On the 2nd postnatal day, all litters were culled to nine pups. Pups remained with their dam for the remainder of the experiment. All animal protocols were approved by the Institutional Animal Care and Use Committee.

Hormone administration. Daily s.c. injections of T_4 at a dose of 130 pmol/g BW (0.1 μg/g BW) were given to pups on postnatal d 5 to 12. This dose has been found to elevate circulating T_4 concentrations 3.5- to 5.5-fold higher than normal at this age (11). The magnitude of this increase is in the same range as the normal developmental rise of serum T_4 (9). The control animals received the same volume of vehicle on d 5 to 12. To demonstrate synergy with T_4, a submaximal dose of DEX had to be used. A DEX dose-response study using s.c. injections in the range 7.5 pmol–12.5 nmol (*i.e.* 0.003–5.0 μg)/g BW/d showed that a dose of 25 pmol (*i.e.* 0.01 μg)/g BW/d caused precocious elevation of sucrase activity to approximately one third the maximum response. In the actual experiment, this dose was administered daily on postnatal d 8 to 12.

Experimental design. Four litters of animals were used for the main study. Each litter was treated as follows: one pup was untreated; one pup received daily vehicle injections on postnatal d 5 to 12; one pup received daily T_4 injections on postnatal d 5 to 12; one pup received daily DEX injections on postnatal d 8 to 12; and one pup received daily T_4 injections on postnatal d 5 to 12 and daily DEX injections on postnatal d 8 to 12. All pups were killed on d 13. Because there were no differences between the uninjected and the vehicle-treated groups, only the vehicle-treated group was used in analysis, as shown in the graphs.

Collection of blood and quantitation of serum corticosterone. Animals were decapitated on postnatal d 13 between 0900 and 1100 h after an overnight isolation period during which the pups and dams were not disturbed. Trunk blood was collected on ice, allowed to clot, and centrifuged at 1500 × *g* for 10 min at 4°C to separate the serum. The serum was then frozen at −10°C for later determination of corticosterone concentration using a competitive protein binding assay as described previously (23).

Collection of tissue. Immediately after collection of blood, the entire small intestine (from the pylorus to the ileocoecal valve) was removed, flushed with ice-cold 150 mM NaCl, separated into duodenum, jejunum, and ileum, and stored at −10°C. The duodenum was defined as the segment proximal to the ligament of Treitz. The jejunum was defined as the proximal half of the segment distal to the ligament of Treitz, and the ileum was defined as the distal half of that segment. As explained earlier, jejunum and ileum were the regions used for assay of the four carbohydrase enzymes, whereas duodenum and ileum were used for assay of alkaline phosphatase.

Enzyme assays. All tissues were thawed and the mucosa was scraped with a glass microscope slide. The mucosa was homogenized in 9 volumes of 154 mM KCl by 15 strokes of a Tri-R homogenizer (Tri-R Instruments, Rockville Centre, NY). Sucrase, lactase, and acid β-galactosidase were assayed as previously described (24, 25) using sucrose, lactose, and ONPG, respectively, as substrates.

Glucoamylase activity was determined by the method of Eggermont (26) with minor modifications. The acid fraction of adult and 13-d-old rat pup mucosal samples was found to account for 1.2–3.6% of the total glucoamylase activity. Because this was negligible, the acid fraction determination at pH 3.0 was omitted from our routine assay. Jejunal and ileal homogenates were diluted 1:2 with deionized H_2O (making a final concentration of 1 g mucosa/20 mL). Enzyme activity in 0.1 mL homogenate was measured by incubating at 37°C with 0.4 mL of substrate solution containing 0.15 M sodium citrate buffer, pH 5.4, 3% (wt/vol) glycogen, and 0.25% (wt/vol) BSA. The reaction was stopped after 60–120 min with $Ba(OH)_2$, then neutralized with $ZnSO_4$. After centrifugation at 2000 rpm for 5 min, the glucose content was measured with glucose oxidase reagent. Glucose oxidase reagent was prepared by dissolving the contents of one bottle of reagent in 100 mL of 1 M Tris buffer, pH 7.3, and making a further 1:2 dilution with the same buffer. To each 1.9 mL of diluted glucose oxidase reagent, 100 μL of supernatant were added and vortexed. All samples were incubated for 10 min and absorbance was read at 505 nm within 60 min.

Alkaline phosphatase activity was determined by the method of Uezato and Fujita (27) with modifications. The original duodenal and ileal homogenates (1 g/10 mL) were further diluted with 139 volumes of deionized H_2O (final dilution 1:1400). Enzyme activity in 0.1 mL homogenate was measured by incubation at 37°C with 0.5 mL 0.2 M Tris buffer, pH 9.8, 0.1 mL 0.1 M $MgCl_2$, and 0.4 mL of 3 mM *p*-nitrophenyl phosphate as substrate. The reaction was stopped at 5 to 10 min with 0.5 mL 2.0 M NaOH. Samples were centrifuged at 4000 rpm for 10 min. The absorbance was read at 400 nm and compared with a standard curve generated with known amounts of *p*-nitrophenol.

Statistics. The data are presented as the mean ± SEM. Results for each enzyme from each intestinal region studied were analyzed using a two-way analysis of variance with DEX and T_4 as the two factors examined. In such an analysis, a significant statistical interaction between the two variables indicates that the response to each hormone is influenced by the presence of the other. Thus, whenever the two hormones influence enzyme activity in the same direction, a significant interaction term in the two-way analysis constitutes a test for synergism between the two hormones. The effects of each hormone in the absence of

the other hormone (*i.e.* vehicle *versus* T_4 and vehicle *versus* DEX) were assessed by Bonferroni-adjusted two-tailed *t* tests. All analyses used a repeated measures design (28) to account for the use of littermates; $p < 0.05$ was considered the limit for statistical significance.

RESULTS

Figure 1 shows the results of hormone treatment on sucrase activity in the jejunum and the ileum. Consistent with the literature, control animals receiving daily vehicle injections showed essentially undetectable sucrase activity at this age (13 d). In both jejunum and ileum, the effects of T_4 alone did not reach statistical significance, whereas DEX alone elicited a clear elevation of sucrase activity. In the jejunum, there was a significant statistical interaction between T_4 treatment and DEX treatment, *i.e.* the effect of the combination of the two hormones was greater than the sum of each hormone administered separately, indicating a synergistic effect on the precocious appearance of sucrase activity in this region. In the ileum, the interaction (*i.e.* synergy) between the two hormones failed to reach statistical significance ($p = 0.068$). Overall, the comparison between the two regions shows a powerful effect of DEX in both regions and a robust synergism between DEX and T_4 in the region (namely, the jejunum) having maximum sucrase activity in the mature intestine (24).

Figure 2 shows corresponding data for glucoamylase activity in the jejunum and ileum. This disaccharidase displayed low activity in control animals in both regions. There was no significant effect of T_4 alone, whereas DEX caused a significant elevation of glucoamylase activity in the ileum but not in the jejunum. Moreover, there was a significant interaction between the two hormones in the ileum, whereas in the jejunum the interaction did not quite reach statistical significance ($p = 0.073$). Overall, the pattern for glucoamylase is very similar to that seen for sucrase, with synergism between the two hormones being clearly demonstrable in the region of higher activity.

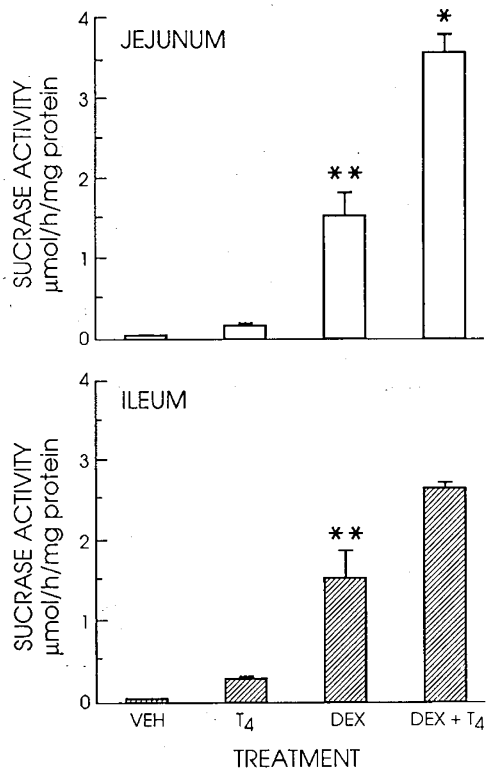

Fig. 1. The effect of T_4, DEX, and DEX + T_4 on the developmental rise of sucrase activity. The *upper panel* shows the results for jejunum, and the *lower panel* shows the results for ileum. In both panels, statistical significances are shown by the following symbols above the bars: *no symbol*, not significant; *, $p < 0.05$; and **, $p < 0.01$. Symbols above the T_4 treatment group show the significance of the effect of T_4 alone, symbols above the DEX group show the effect of DEX alone, and those above the DEX + T_4 group show the interaction between DEX and T_4. Values are given as means ± SEM ($n = 4$).

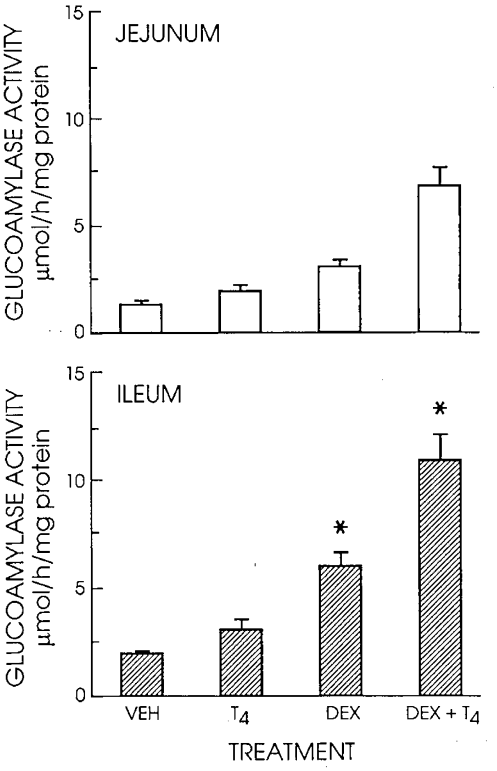

Fig. 2. The effect of T_4, DEX, and DEX + T_4 on the developmental rise of glucoamylase activity in jejunum (*upper panel*) and ileum (*lower panel*). Statistical significances indicated as in Figure 1.

Figure 3 shows the results of hormone treatment on alkaline phosphatase activity in the duodenum and the ileum. As expected, in control animals aged 13 d, alkaline phosphatase activity was low in the duodenum and high in the ileum. In the duodenum (where the enzyme activity normally increases during development), although neither hormone alone had a significant effect, when administered together they displayed significant synergism and caused a precocious increase of enzyme activity. In contrast, in the ileum, where the enzyme activity normally declines during development, all treatments were without significant effect. Thus, once again we see synergism between DEX and T_4 in the region that displays maximal activity in the mature animal (20).

Figure 4 shows the data for lactase activity in the jejunum and ileum. Recall that this enzyme normally declines during the 3rd postnatal week in both these regions, although more steeply in the ileum (19). Thus, control animals at 13 d of age displayed high lactase activity in both jejunum and ileum. In both regions, neither T_4 alone nor DEX alone yielded a statistically significant effect on lactase activity. Similarly, interaction between the two hormones was not significant in either region ($p = 0.067$ and $p = 0.117$ for jejunum and ileum, respectively). Note that even if the interaction had reached statistical significance, for this enzyme it would not have constituted synergism (but rather interference) because the trends for the separate effects of the two hormones were in opposite directions.

Figure 5 shows the results for acid β-galactosidase, another enzyme that normally declines in activity during the 3rd postnatal week. This lysosomal enzyme is predominantly expressed in the ileum of the suckling rat, as indicated here by the data for control animals. Although all hormone treatments tended to

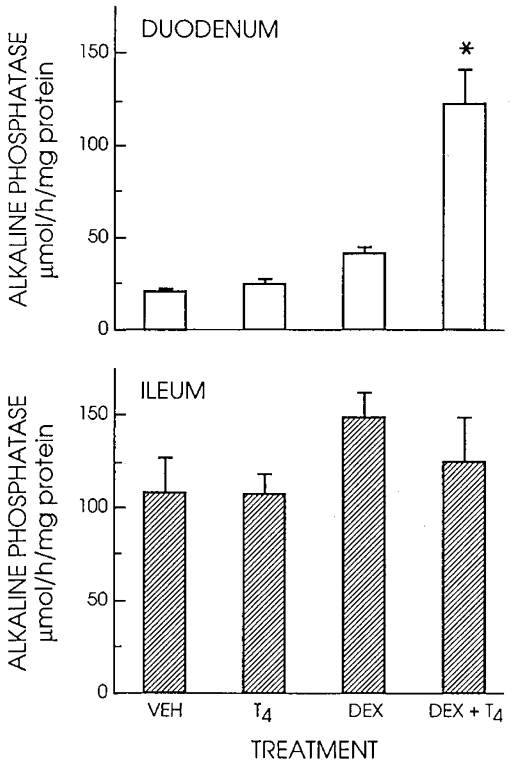

Fig. 3. The effect of T_4, DEX, and DEX + T_4 on the developmental rise of duodenal alkaline phosphatase activity (*upper panel*) and on the developmental decline of ileal alkaline phosphatase (*lower panel*). Statistical significances indicated as in Figure 1.

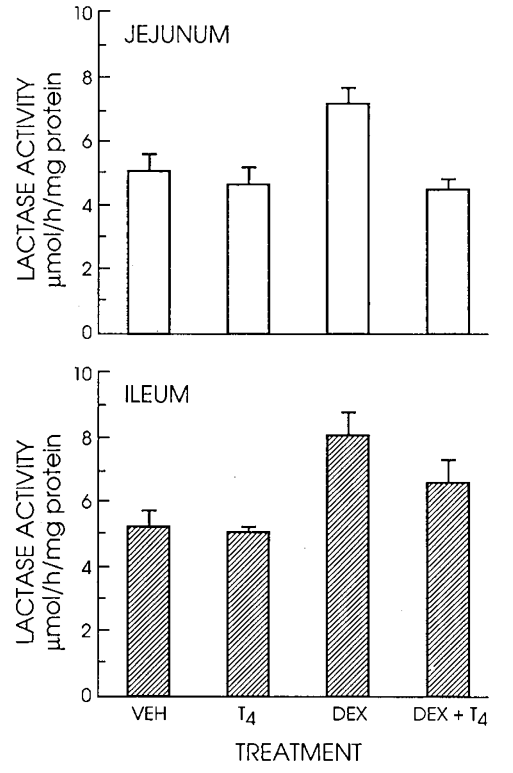

Fig. 4. The effect of T_4, DEX, and DEX + T_4 on the developmental decline of lactase activity in jejunum (*upper panel*) and ileum (*lower panel*). Statistical significances indicated as in Figure 1.

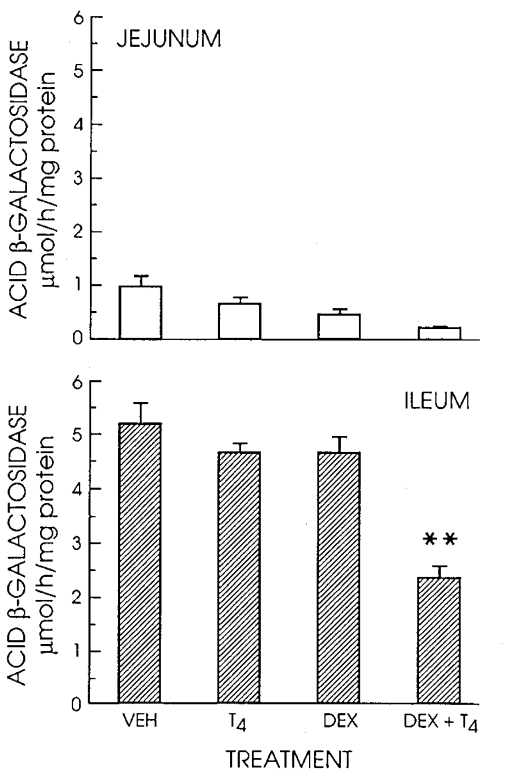

Fig. 5. The effect of T_4, DEX, and DEX + T_4 on the developmental decline of acid β-galactosidase activity for jejunum (*upper panel*) and ileum (*lower panel*). Statistical significances indicated as in Figure 1.

cause a precocious decrease in activity, the effects of neither T_4 alone nor DEX alone reached statistical significance. In the ileum, the two hormones interacted synergistically to cause a precocious decline of acid β-galactosidase activity. There was no significant interaction in the jejunum ($p = 0.64$). Overall, the pattern for acid β-galactosidase is the same as that seen for sucrase, glucoamylase, and alkaline phosphatase, with DEX and T_4 synergizing to cause precocious maturation in the region of maximal enzyme activity.

As explained earlier, the design of these experiments was carefully chosen to assure sound conclusions regarding synergism between T_4 and glucocorticoids. Specifically, DEX was used to avoid T_4 influencing the circulating concentration of the exogenous glucocorticoid. Because T_4 also elevates blood levels of endogenous corticosterone, to check for the possibility of apparent synergism being confounded by this factor it was essential to measure serum corticosterone in all animals. The data are shown in Figure 6. The sucrase data from Figure 1 also are reproduced for comparison. Serum corticosterone was extremely low in the control animals (as expected for this age) and was below the limit of detection in the DEX group. Administration of T_4 alone caused a significant elevation of circulating corticosterone. There was also a significant interaction between DEX and T_4 so the concentration of corticosterone was substantially lower in animals receiving T_4 and DEX, compared with animals receiving T_4 alone. Comparison of the corticosterone data with the jejunal sucrase activities in the same animals shows obvious differences in the response patterns of these two parameters. For example, although T_4 alone markedly increased endogenous corticosterone concentrations, it had no significant effect on jejunal sucrase activity. In comparison, animals receiving the combined hormone treatment displayed significantly lower concentrations of corticosterone but higher jejunal sucrase activity. This comparison of enzyme activity with serum corticosterone concentration was made for all five enzymes studied in both regions of the small intestine; no significant correlation was found.

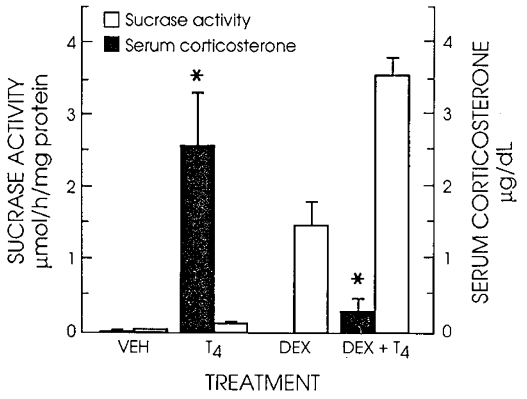

Fig. 6. Total serum corticosterone concentration *versus* jejunal sucrase activity for the VEH (daily vehicle injection), T_4, DEX, and DEX + T_4 treatment groups. *Black bars* denote total serum corticosterone (1 μg/dL = 29 nM), and *open bars* denote jejunal sucrase activity (data repeated from Figure 1). Statistical significances for serum corticosterone are indicated as in Figure 1.

Table 1. *Summary of enzyme ontogeny and response to T_4, DEX, and DEX plus T_4**

Enzyme	Region	Ontogeny	T_4	DEX	DEX + T_4
Sucrase	Jejunum	↑	–	↑	↑↑
	Ileum	↑	–	↑	↑
Glucoamylase	Jejunum	↑	–	–	↑
	Ileum	↑	–	↑	↑↑
Alkaline phosphatase	Duodenum	↑	–	–	↑↑
	Ileum	↓	–	–	–
Lactase	Jejunum	↓	–	–	–
	Ileum	↓	–	–	–
Acid β-galactosidase	Jejunum	↓	–	–	↓
	Ileum	↓	–	–	↓↓

* Ontogeny refers to the normal development of these small intestinal enzymes: ↑, increases with postnatal maturation; ↓, decreases with postnatal maturation. Symbols under each of the hormone treatments used in our study refer to the measured change in enzyme activity: –, no change; ↑, increase in activity; ↓, decrease in activity; ↑↑ and ↓↓, synergistic response to DEX + T_4.

DISCUSSION

Although a number of previous studies have examined the possibility of a synergism between T_4 and GC in the regulation of small intestinal enzyme ontogeny (8, 17, 18, 29–31), they all have been compromised for various reasons. The three principal problems have been *1*) the use of pharmacologic doses of T_4 (17, 29, 31), *2*) the use of hydrocortisone or other steroids that bind CBG (8, 18, 31), and *3*) the use of saturating doses of DEX (30). In the current study, T_4 was used in a dose known to be physiologic, and DEX was chosen as the GC to avoid the changes in metabolic clearance rate elicited by changes in the concentration of circulating CBG. In addition, a dose-response study was conducted initially to determine the dose of DEX that induced less than a 50% increase in sucrase activity. In this way, synergy with T_4 could still be observed. Under these carefully prescribed conditions, we have confirmed that T_4 and DEX synergize in eliciting early maturation of four of the five enzymes studied (Figs. 1–5). These findings using exogenous hormones strongly suggest that during normal ontogeny, the rising concentrations of T_4 and corticosterone act synergistically to elicit the enzyme changes that occur in the rodent intestine during the 3rd postnatal week. Analogous synergism between these two hormones has been found during prenatal lung development (32) and during postnatal liver development (33).

The findings of this investigation show that each of the five enzymes studied has a unique developmental pattern and response to hormonal manipulation. It is not possible to draw generalized conclusions regarding enzyme development based on the behavior of a single enzyme in a single region of the small intestine. From the summary of our findings in Table 1, it can be seen that those enzymes that rise postnatally responded synergistically to DEX + T_4, but those enzymes that decline postnatally showed a mixed response to DEX + T_4. The lysosomal enzyme, acid β-galactosidase, responded synergistically to DEX + T_4 distally, although the brush-border enzyme, lactase, did not. Our findings with alkaline phosphatase are particularly interesting. In the duodenum, where activity increases during development (20), the response to DEX and T_4 essentially mimicked that of sucrase and glucoamylase. In contrast, in the ileum, where activity decreases with development (19), alkaline phosphatase behaved like lactase, showing no response to the hormone manipulations.

A number of studies have suggested that lactase maturation may involve different mechanisms than do other brush-border enzymes (17, 19, 22, 31, 34–36). For example, recent studies have reported the detection of significant levels of lactase mRNA in the adult intestine despite a decline in lactase catalytic activity (36). This is in contrast to sucrase enzyme activity, which coincides with its mRNA production (24). In addition, posttranslational sites of regulation for lactase have been proposed, such as increased degradation of the active lactase subunit, a switch in lactase synthesis to an inactive lactase subunit in the adult intestine, and a shortened life span of the mature lactase-bearing enterocyte (34, 35). To date, there have been no studies of the cellular and molecular mechanisms involved in the developmental decline of ileal alkaline phosphatase activity. Given the striking similarity between lactase and ileal alkaline phosphatase in our study, it will be of interest to assess the full extent to which these enzymes share common mechanisms for the regulation of their ontogeny.

Treatment with physiologic doses (130 pmol/g BW, *i.e.* 0.1 μg/g BW) of T_4 alone had modest effects on enzyme maturation that failed to reach our criteria for statistical significance. Studies with a larger *n* or with fewer comparisons (thus obviating the need for the Bonferroni correction) were not deemed worthwhile, however, because previous studies have shown that effects of the same dose of T_4 alone in intact animals are largely the result of the consequent elevation of circulating corticosterone (16) and thus should be described as "apparent effects of T_4 alone." The data in Figure 6 corroborate this point by showing that serum corticosterone was significantly elevated in animals receiving T_4 alone. Other studies in which pharmacologic doses of T_4 have been found to affect intestinal development in intact animals (8, 37–42) are confounded by even greater increases of circulating corticosterone (11). Even studies in adrenalectomized (16, 43) or hypophysectomized (44) animals are suspect unless circulating corticosterone has been measured (16). In contrast, our conclusions regarding synergy between DEX and T_4 are sound because the data in Figure 6 show clearly that T_4, when given with DEX, was unable to elevate serum corticosterone under these conditions (presumably because of the negative feedback effect of DEX on the hypothalamo-pituitary-adrenal axis).

The actual mechanism through which T_4 influences the small intestine has yet to be determined. The finding that T_4 does not synergize with GC to elicit maturation of intestinal explants (10) could be taken as evidence that *in vivo*, T_4 does not act directly on the intestine but rather via some other organ system. On the other hand, the explant data must be interpreted with caution, because T_4 receptors that are clearly present on enterocytes *in vivo* (45) may have been lost during culture. Because GC have been shown to exert their effects on intestinal development via the proliferating cells in the intestinal crypts (46, 47), the most likely explanation for the synergy between GC and T_4 is faster emergence of cells from the crypts in response to T_4 (acting either directly or indirectly). Yeh *et al.* (48) recently reported faster emergence using pharmacologic doses of T_4 (1.3 nmol/g BW,

i.e. 1 μg/g BW). Our future goals are to observe the rate of cell emergence from the crypts under physiologic conditions (T_4 at 130 pmol/g BW, *i.e.* 0.1 μg/g BW) and to detect the mRNA for each enzyme by using *in situ* hybridization. The possibility that DEX and T_4 act directly on the genes for intestinal enzymes (via their respective receptors and regulatory elements) to influence synergistically the rate of transcription also warrants investigation as the appropriate molecular tools become available.

Acknowledgments. The authors thank Lucy Leeper and Nanda Nanthakumar for their encouragement and support with this project, Rob Shulman for his helpful comments on the manuscript, and E. O'Brian Smith and Laura Ferlic for the statistical analyses.

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