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Thyroxine-Evoked Precocious Decrease of Acid Hydrolases in the Ileum of Suckling Rats[1] (38167)

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(Introduced by A. S. Goldman)

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The activity of several acid (lysosomal) hydrolases in the small intestine, especially ileum, of various mammals during the suckling period is high. During the weaning period these activities decrease (1–7).

In experiments performed with rats the adrenals were identified as at least one factor responsible for this decrease. Administration of cortisone acetate (8, 9) or hydrocortisone sodium phosphate (unpublished results) to suckling rats caused a precocious drop of activity of acid hydrolases; on the other hand adrenalectomy performed on day 14 postnatally delayed this decrease (3, 10, 11).

It is well established that the thyroid gland influences maturation of various functions in different organs during the suckling period (12–14). As far as the development of the small intestine is concerned, there are only two reports (15, 16) studying the regulatory role of this endocrine gland. Chan *et al.* (15) have found that administration of thyroxine to suckling rats caused premature cessation of macromolecular uptake by the intestine. Yeh and Moog (16) have just recently described that thyroidectomy performed during the suckling period in rats prevents the usual decline of lactase activity; administration of thyroxine normalized this decrease.

In the present communication we report results indicating the involvement of thyroid in the regulation of the decrease of activity of acid hydrolases in the ileum.

Materials and methods. Pregnant Charles River rats were obtained and gave birth in our own animal house. On the second day after birth, the size of the litter was reduced to 8–9 pups. Adrenalectomy was performed during the morning hours via the dorsal route under ether anaesthesia and suckling rats were than returned to their mother; they remained together with her until their sacrifice. They received 0.9% NaCl instead of tap water in drinking bottles. D-L-Thyroxine (Sigma, St. Louis, Mo.), dissolved in 0.005 *N*-NaOH was injected s.c. (200 μg/100 g body wt) every 24 hr; controls received only 0.005 *N* NaOH (0.5 ml/100 g body wt). Animals were sacrificed by decapitation and the small intestine was excised. The duodenum was discarded. The remaining small intestine was divided along its length into 3 segments (10), the first being called the jejunum, the last the ileum. After flushing the segments with ice-cold 0.9% NaCl, the segments were homogenized in bi-distilled water in a Potter–Elvehjem homogenizer using a Teflon piston.

Assay of acid-β-galactosidase, *N*-acetyl-β-glucosaminidase and β-glucuronidase was performed as described in our previous communications (4, 8, 9). The determinations were made under conditions of linearity of activity with time and amount of enzyme. Composition of assay mixtures is summarized in Table I. Assay of sucrase and maltase activities was performed according to Dahlqvist (17) in a slightly modified

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TABLE I. Characteristics of Glycosidases Studied.

Enzyme	Acid β-galactosidase	*N*-acetyl-β-glucosaminidase	β-Glucuronidase
Substrate[a]	*P*-Nitrophenyl-β-galactoside	*P*-Nitrophenyl-*N*-acetyl-β-glucosaminide	*P*-Nitrophenyl-β-glucuronide
Final concentration of substrate	5 m*M*	2.5 m*M*	5 m*M*
Buffer used[b]	Na—citrate	Na—citrate	Na—acetate
pH of the buffer	3.5	4.5	4.0

[a] All from Calbiochem Co., San Diego, Calif.

[b] Buffers contained Triton X-100 (Rohm & Haas, Philadelphia, Pa.) in a final assay concentration of 0.1% and bovine albumin (Pentex, Kankakee, Ill.) in a final assay concentration of 1 mg/ml.

form as described in our previous communications (18, 19). Protein was determined according to Lowry *et al.* (20). Student's *t*-test was employed to determine the significance of the results. Since no sex differences were observed, data from both sexes were pooled.

Results. In the first experiment thyroxine was injected daily to suckling rats starting day 7 postnatally and they were then killed on day 13. In agreement with previous data (12, 13) thyroxine-treated rats, when compared to control animals, gained less weight and the appearance of their fur was more mature. Their eyes were already open on day 13, although not in controls. The activity of the three studied lysosomal hydrolases decreased precociously in thyroxine-treated rats (Table II).

To exclude the possibility of stimulation of the adrenals by application of thyroxine, further experiments were performed on adrenalectomized rats. This precaution was especially important in light of the experiments of Schapiro and Norman (20) who showed that administration of thyroxine to newborn rats accelerated development of their adrenal corticosterone response to stress.

Rats were adrenalectomized on day 14 postnatally. Some then received thyroxine while others were given solvent only. The results of this experiment are summarized in Table III.

Administration of thyroxine to adrenalectomized 14-day-old rats did not influence the body weight when compared to controls; however it did cause a decrease in the weight of the ileum (both expressed as total mg of protein and as total mg of wet weight-data not given). As in the previous experiment (Table II), thyroxine again evoked a substantial decrease of activity of all three acid hydrolases whether expressed as per mg protein or as total activity per ileum per rat.

Discussion. Injection of thyroxine caused a precocious decrease of activity of all three acid hydrolases studied in the ileum of suckling rats. This effect was not secondary to stimulation of the adrenals, since it was also found in adrenalectomized suckling rats. Thus, in addition to the already established effect of corticosteroids on these enzymes, we have found that a hormone of another endocrine gland—thyroid gland—is also able to influence them during the suckling period.

This conclusion is in concert with a known general maturative effect of both hormones on various functions of other organs during the mammalian perinatal period. Our data, together with previously mentioned studies (15, 16) indicate the maturative role of the thyroid in the development of the small intestine. This conclusion is further substantiated by observed changes of sucrase and maltase activities in the jejunum of rats used in our experiments. These enzymes, which have a low activity in suckling rats and then increase during the weaning period (22), were precociously elevated toward adult values by thyroxine treatment (Sucrase: rats from experiment given in Table

TABLE II. Effect of Thyroxine on Activity of Acid-β-Galactosidase, *N*-Acetyl-β-Glucosaminidase and β-Glucuronidase in the Ileum of 13-day-old Rats.*

Group	Number of animals	Body weight (g)	Weight of the ileum (mg protein)	Activity (μmol/mg protein/60 min) Acid β-galactosidase	*N*-acetyl-β-glucosaminidase	β-glucuronidase
Control	4	30 ± 0.4	32.4 ± 1.5	6.2 ± 0.23	19.7 ± 0.77	2.6 ± 0.11
Thyroxine-treated	5	26 ± 0.2	29.2 ± 2.1	1.0 ± 0.21	2.6 ± 0.41	0.4 ± 0.04
Level of significance between groups		0.001	0.3 (not significant)	0.001	0.001	0.001

* Injections performed daily started on day 7 when weight of rats in both groups was 15.0 + 0.1 g. All values are given as mean ± S.E.M.

TABLE III. Effect of Daily Injection of Thyroxine on Activity of Acid β-Galactosidase, *N*-Acetyl-β-Glucosaminidase and β-Glucuronidase in the Ileum of 18-day-old Rats Adrenalectomized on Day 14.

Group	Number of animals	Body weight (g)	Weight of ileum (mg of protein)	Activity (μmol/mg protein/60 min) Acid β-galactosidase		*N*-acetyl-β-glucosaminidase	β-glucuronidase
Control	14	38.6 ± 1.6	44.0 ± 3.2	4.56 ± 0.53	(197 ± 22) [a]	16.8 ± 1.47	1.83 ± 0.26
Thyroxine-treated	12	37.2 ± 1.1	34.0 ± 1.6	1.92 ± 0.25	(63 ± 8)	7.3 ± 0.72	0.82 ± 0.10
Level of significance between groups		0.5 (not significant)	0.01	0.001	0.001	0.001	0.01

* Rats were operated on day 14 (body weight in both groups was 29.9 ± 0.4 g) and injected s.c. daily with thyroxine (200 μg/100 g B.W.). All values are given as mean ± S.E.M. Total activities in the ileum given as μmol/60 min.

II: controls: 0.097± (μmol of glucose liberated per mg protein/60 min), thyroid treated: 2.54 ± 0.15; rats from experiment in Table III: controls: 0.41 ± 0.57, thyroxine treated: 1.99 ± 0.20. Maltase values (in the same order) were 9.15 ± 1.60 and 48.18 ± 3.65; 6.0 ± 0.33 and 19.5 ± 1.62.

The doses of thyroxine employed in our experiments (similar to those used in other laboratories—13, 15, 16, 21) are quite high and have to be judged as pharmacological. Further experiments are therefore needed to elucidate both the mechanisms of thyroxine action and the role of thyroid under normal physiological circumstances on the maturation of the mentioned intestinal functions.

The role of the lysosomal enzymes in the small intestine during the suckling period is not clear. It has been suggested that the lysosomes are involved in the process of a high uptake of macromolecules occurring during this period (23–26). Interestingly, both corticosteroids and thyroxine precociously decreased the activity of acid hydrolases and at the same time both caused a precocious decrease of macromolecular uptake in the small intestine in rats (15, 26). Last but not least our data should be added as a further contribution to the so far neglected problem of hormonal regulation of activity of lysosomal enzymes as such during perinatal development of mammals.

Summary. Daily administration of thyroxine (200 μg/100 g/BW 24 hr s.c.) to intact suckling rats (from 7 to 13 days of postnatal age) and to suckling adrenalectomized rats (from 14 to 18 days) caused a precocious decrease of acid-β-galactosidase, β-glucuronidase and *N*-acetyl-β-glucosaminidase in the ileum of the small intestine. Thyroxine treatment evoked also a precocious increase of jejunal sucrase and maltase activities.

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