Effect of Cortisone on the Developmental Pattern of the Neutral and the Acid β -Galactosidase of the Small Intestine of the Rat

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(Received 4 November 1969)

1. The developmental pattern and effect of cortisone on acid β -galactosidase and neutral β -galactosidase were studied in postnatal rats by a recently proposed method for their independent determination. 2. After birth the acid β -galactosidase activity increases in the ileum, whereas it decreases slightly in the jejunum. On day 16 after birth the activity in the ileum decreases and in 20-day-old rats activity in both parts of the intestine decreases to adult values. In suckling animals the activity in the ileum exceeds the jejunal activity severalfold and in adult animals the activity in the jejunum is slightly higher than that in the ileum. 3. Neutral β -galactosidase activity is high after birth and decreases in both jejunum and ileum after day 20 after birth. In 12-20-day-old rats activity in both parts is essentially the same, but in adult animals jejunal activity exceeds ileal activity four- to five-fold. 4. Cortisone (0.5, 2.0 or 5.0 mg/100 g body wt. daily for 4 days) does not influence the activity of either enzyme in 60-day-old rats. Acid β -galactosidase activity is decreased after cortisone treatment in 8-, 12-, 16- and 18-day-old rats, with sensitivity to cortisone increasing with the approach of weaning. No effect of cortisone on acid β -galactosidase is seen in 8-day-old rats. Neutral β -galactosidase activity is increased in the ileum of 8-, 12-, 16- and 18-day old rats, but only in the jejunum of 8- and 12-day-old rats.

Recent investigations have demonstrated that the enterocytes of the small intestine of the rat contain at least two enzymes with β -galactosidase activity (Asp & Dahlqvist, 1968*a,b*; Alpers, 1969; Kraml *et al.* 1969). Although the two enzymes have some common characteristics, such as a capacity to hydrolyse lactose, *o*-nitrophenyl β -galactoside and *p*-nitrophenyl β -galactoside (Asp & Dahlqvist, 1968*b*), and being present in higher activity during the suckling period (Doell & Kretchmer, 1962; Koldovský & Chytil, 1965; Rubino, Zimbalatti & Auricchio, 1964), they differ in several respects and have different localizations within the cell. One enzyme with a pH optimum at 5.5-6.0 (neutral β -galactosidase) and located only in the microvilli (Koldovský *et al.* 1965*b*; Alpers, 1969), is very active in the cells of the upper part of the villi and is virtually absent from the cells of the crypts (Nordström, Koldovský & Dahlqvist, 1969). The second enzyme (acid β -galactosidase) has a pH optimum at 3.0-4.0, hydrolyses not only the three substances mentioned above but also 6-bromo-2-

naphthyl β -galactoside and phenyl β -galactoside and is located in the lysosomal fraction of the enterocytes (Alpers, 1969). The acid enzyme, which exhibits substantial activity at pH 5.5, interferes with the determination of the activity of the neutral β -galactosidase. The discovery that *p*-chloromercuribenzoate inhibits the activity of the acid β -galactosidase, but has no effect on the activity of the neutral β -galactosidase, allowed the development of a method for the specific determination of the neutral β -galactosidase in the presence of the acid enzyme (Koldovský, Asp & Dahlqvist, 1969).

The present studies compare the normal developmental pattern of the two enzymes in both the jejunum and ileum of rats, and evaluate the effect of cortisone on these patterns. These are extensions of previous studies in which it was demonstrated that adrenalectomy could delay the decrease in activity of acid β -galactosidase that normally occurs during the weaning period (Koldovský & Chytil, 1965), and that the effect of adrenalectomy at weaning could be prevented by the administration of corticosterone (Koldovský, Jirsová & Heringová, 1965*a*).

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MATERIALS AND METHODS

White rats of the Wistar strain were used. Litters were decreased to eight animals on day 3 after birth and were used regardless of sex. They were fed on a standard pellet diet (Purina Laboratory Chow) and were weaned by day 28 after birth.

Preparation of tissue. Fed animals were decapitated. Their intestines were rinsed with ice-cold 0.15M-KCl and removed. The duodenum was discarded and the rest of the small intestine was divided into three equal parts. The proximal third was used as jejunum and the distal third as ileum. The individual thirds were rinsed with ice-cold 0.15M-NaCl and dried with filter paper. They were weighed on a torsion balance and then homogenized in 2ml of ice-cold water in a Potter-Elvehjem homogenizer with a Teflon piston. In the animals that were 60 days old the mucosa from the jejunum and ileum was removed with a spatula and used instead of whole intestine.

Cortisone. Each litter was divided into four pairs of rats; one pair was untreated and served as controls; the other three were given daily intramuscular injections of cortisone acetate (Upjohn Co., Kalamazoo, Mich., U.S.A.). One pair received 0.5mg, the second pair received 2.0mg and the third received 5.0mg/100g body wt. The cortisone was diluted in water so that each animal received 1 ml of fluid/100g body wt. daily. Injections were started 5 days before the day on which the animals were killed.

Determination of protein. The method of Lowry, Rosebrough, Farr & Randall (1951) was used. A fresh serum albumin solution served as a standard.

Enzymic assay. The activities of the neutral β -galactosidase and the acid β -galactosidase were determined in homogenates as described by Koldovský *et al.* (1969). In each assay the reaction mixture contained 0.5ml of homogenate and 0.5ml of 48mM-*o*-nitrophenyl- β -galactoside (Calbiochem, Los Angeles, Calif., U.S.A.). For the determination of the acid β -galactosidase 1.0ml of 0.1M-sodium citrate buffer, pH3.5, was used, and for the assay of the neutral β -galactosidase 1ml of 0.1M-sodium citrate buffer, pH5.5, containing 0.2mM-*p*-chloromercuribenzoate (if homogenates contained more than 1mg wet wt./ml the concentration of *p*-chloromercuribenzoate was increased to 0.8mM). The incubation was carried out at 37°C for 60 min. The reaction was terminated and the colour developed by the addition of 1.0ml of tris-HCl buffer, pH9.0, if the incubation had been performed at pH3.5, or tris-HCl buffer, pH8.5, if the incubation had been performed at pH5.5. Blanks were prepared in a similar manner, but the substrate was added after the incubation. The extinction at 420nm was measured, and the amount of *o*-nitrophenol liberated was calculated from a standard curve prepared with free *o*-nitrophenol. The enzymic activity was expressed as μ mol of substrate hydrolysed/60 min per mg of protein.

Statistical evaluation. The *t* test was used.

RESULTS

Acid β -galactosidase. In newborn rats that had not yet consumed milk, the specific activity of the acid β -galactosidase was similar in both the jejunum

Fig. 1. Postnatal developmental activities of β -galactosidases: $\circ$, jejunum; $\triangle$, ileum. Mean values are presented; vertical lines denote $2 \times$ S.E.M. $\blacktriangle$ denotes that the difference between the values for the jejunum and ileum of that particular age group is statistically significant ($P < 0.01$).

and ileum (Fig. 1a). The activity then increased rapidly in the ileum, but decreased slightly in the jejunum. On the day 16 after birth the activity in the ileum began to decrease and by day 22 low adult values were found in both portions of the small intestine with the activity being slightly lower in the jejunum than in the ileum. These findings are similar to the developmental pattern seen in other laboratories with a different line of Wistar rats (Koldovský & Chytil, 1965).

Neutral β -galactosidase. The activity of the neutral β -galactosidase in both jejunum and ileum was greater in the suckling period than in later life (Fig. 1b). Between day 20 and day 25 after birth the activity in both areas fell rapidly to reach adult values. The difference in the activity of the neutral β -galactosidase between the jejunum and ileum was greatest in adult animals, where the ileal activity represented about 20% of the jejunal activity; the difference was also marked in the newborn period, but there was no difference in activity in the 12–20-day-old rats. This decrease in activity of the neutral β -galactosidase towards adult values occurred about 5 days later than did the decrease in activity of the acid β -galactosidase.

Effect of cortisone. The effect of cortisone was studied in rats 8, 12, 16, 18 and 60 days old.

Cortisone did not affect the activity of acid β -galactosidase in 8- and 60-day-old animals. When

5mg of cortisone/100g body wt. was injected there was a decrease in activity in the acid β -galactosidase in both the jejunum and ileum of 12-, 16- and 18-day-old animals (Table 1). When 2mg of cortisone/100g body wt. was given a decrease in activity was noted in 16- and 18-day-old rats; when 0.5mg of cortisone/100g body wt. was used a decrease was noted only in the 18-day-old animals. Cortisone exerted a greater effect on the activity of

the enzyme in the ileum as the animal approached the weaning period. When the activities in the ileum after cortisone administration were expressed as a percentage of the activities of control animals in each group, it was found that 0.5mg of cortisone was more effective in 18-day-old than in 8-day-old animals; the 2.0mg dose was more effective in 16- and 18-day-old than in 8- and 12-day-old animals; and the 5.0mg dose decreased the activity in each age group (Fig. 2).

Cortisone increased the activity of the neutral β -galactosidase in the jejunum of 8- and 12-day-old rats, but did not affect the activity in older rats. In the ileum the activity was increased after cortisone treatment in 8-, 12-, 16- and 18-day-old rats. No effect was seen in 60-day-old rats (Table 2).

DISCUSSION

The activities of the acid β -galactosidase and neutral β -galactosidase differ considerably in the various areas of the small intestine during development. The activities of both enzymes are greater in the suckling period and decrease towards adult values during the period of weaning. The activity of acid β -galactosidase increases markedly in the ileum after birth to reach peak values between day 8 and day 16 of life and then decreases over a 5-day period to reach adult values of 20–21 days after birth. Neutral β -galactosidase is very active at birth, and at 20 days the activity is still above the adult value. After a 5-day period the activity decreases to reach adult values. There is a substantial difference between the activities of neutral β -galactosidase in the jejunum and the ileum in

Fig. 2. Effect of cortisone on the activity of acid β -galactosidase in the ileum of suckling rats. The mean values of the control (no cortisone) groups at each age were taken as 100%, and the individual activities are expressed as a percentage of this mean value. Vertical lines denote $2 \times$ s.e.m. for each group. At least six animals were used, and 0.5mg (○), 2.0mg (△) and 5.0mg (□) denote the doses of cortisone per 100g body wt. that was administered daily. Full symbols denote $P < 0.01$; half-full symbols denote $P < 0.02$ (against control values).

Table 1. *Effect of cortisone on the acid β -galactosidase in jejunum and ileum of rats of different ages*

Doses of cortisone are expressed as the daily dose per 100g body wt. Values that are statistically significantly different from the control values are indicated by ** $P < 0.01$; * $P < 0.05$. The animals had been treated with cortisone for 4 days before the given age that they were killed. Values are expressed as mean (μ mol of substrate hydrolysed/60 min per mg of protein) $\pm$ s.e.m. (number of animals); the same number of animals were used in the determinations for the ileum as are denoted for the jejunum.

Age (days)	Dose of cortisone	...	None	0.5mg	2.0mg	5.0mg
Jejunum						
8			0.73 $\pm$ 0.065 (7)	0.83 $\pm$ 0.075 (7)	0.74 $\pm$ 0.075 (5)	0.62 $\pm$ 0.034 (7)
12			0.44 $\pm$ 0.03 (9)	0.43 $\pm$ 0.031 (5)	0.26 $\pm$ 0.028 (4)*	0.23 $\pm$ 0.032 (6)**
16			0.68 $\pm$ 0.071 (11)	0.67 $\pm$ 0.055 (6)	0.37 $\pm$ 0.034 (5)**	0.31 $\pm$ 0.037 (11)**
18			0.46 $\pm$ 0.084 (6)	0.14 $\pm$ 0.007 (6)	0.25 $\pm$ 0.01 (4)*	0.18 $\pm$ 0.023 (6)**
60			0.13 $\pm$ 0.008 (6)	0.14 $\pm$ 0.007 (6)	0.13 $\pm$ 0.005 (6)	0.14 $\pm$ 0.003 (6)
Ileum						
8			7.3 $\pm$ 0.75 (7)	8.0 $\pm$ 0.91 (7)	6.8 $\pm$ 0.39 (5)	7.9 $\pm$ 0.81 (7)
12			7.2 $\pm$ 0.14 (9)	6.7 $\pm$ 0.35 (5)	5.1 $\pm$ 1.0 (4)	3.1 $\pm$ 0.4 (6)**
16			7.5 $\pm$ 0.35 (11)	5.8 $\pm$ 1.25 (6)	2.1 $\pm$ 0.46 (5)*	1.42 $\pm$ 0.22 (11)**
18			6.5 $\pm$ 1.26 (6)	4.1 $\pm$ 1.05 (6)	1.2 $\pm$ 0.25 (4)**	0.46 $\pm$ 0.083 (6)**
60			0.14 $\pm$ 0.003 (6)	0.25 $\pm$ 0.005 (6)	0.19 $\pm$ 0.004 (6)	0.20 $\pm$ 0.006 (6)

Table 2. *Effect of cortisone on the neutral β -galactosidase in jejunum and ileum of rats of different ages*

Doses of cortisone are expressed as the daily dose per 100 g body wt. Values that are statistically significantly different from the control values are indicated by: ** $P < 0.01$; * $P < 0.05$. The animals had been treated with cortisone for 4 days before the given age that they were killed. Values are expressed as mean (μmol of substrate hydrolysed/60 min per mg protein) $\pm$ S.E.M. (number of animals); the same number of animals were used in the determinations for the ileum as are denoted for the jejunum.

Age (days)	Dose of cortisone ...	None	0.5 mg	2.0 mg	5.0 mg
Jejunum					
8		1.01 $\pm$ 0.14 (7)	1.43 $\pm$ 0.24 (7)	1.62 $\pm$ 0.12 (5)**	1.54 $\pm$ 0.15 (7)**
12		0.82 $\pm$ 0.046 (9)	1.30 $\pm$ 0.31 (5)	1.22 $\pm$ 0.12 (4)**	1.28 $\pm$ 0.14 (6)**
16		0.75 $\pm$ 0.04 (11)	0.77 $\pm$ 0.13 (6)	0.76 $\pm$ 0.08 (5)	0.75 $\pm$ 0.06 (11)
18		0.65 $\pm$ 0.09 (6)	0.64 $\pm$ 0.08 (6)	0.64 $\pm$ 0.08 (4)	0.65 $\pm$ 0.04 (6)
60		0.20 $\pm$ 0.017 (6)	0.20 $\pm$ 0.02 (6)	0.28 $\pm$ 0.021 (6)	0.28 $\pm$ 0.022 (6)
Ileum					
8		0.80 $\pm$ 0.08 (7)	1.12 $\pm$ 0.011 (7)*	1.58 $\pm$ 0.02 (5)**	1.79 $\pm$ 0.03 (7)**
12		0.80 $\pm$ 0.06 (9)	1.38 $\pm$ 0.23 (5)*	1.48 $\pm$ 0.27 (4)*	1.72 $\pm$ 0.27 (6)**
16		0.61 $\pm$ 0.04 (11)	0.79 $\pm$ 0.14 (6)	0.72 $\pm$ 0.08 (5)	1.35 $\pm$ 0.29 (11)*
18		0.80 $\pm$ 0.08 (6)	1.16 $\pm$ 0.08 (6)*	1.14 $\pm$ 0.25 (4)	1.08 $\pm$ 0.08 (6)*
60		0.048 $\pm$ 0.007 (6)	0.024 $\pm$ 0.004 (6)	0.050 $\pm$ 0.005 (6)	0.032 $\pm$ 0.006 (6)

adult animals, whereas essentially no difference exists in the activity of the acid β -galactosidase.

These findings suggest that function may change in the various portions of the small intestine during development (Koldovský & Chytil, 1965; Heringová, Jirsová & Koldovský, 1965; Pelichová, Koldovský, Heringová, Jirsová & Kraml, 1967; Koldovský, 1969; Batt & Schachter, 1969). We might speculate that the digestion of lactose occurs along the entire length of the small intestine during the suckling period, whereas in the newly born and in the adult animals the digestion of lactose is more restricted to the upper portions of the small intestine (Newey, 1967).

The effect of cortisone on the developmental pattern of the two intestinal β -galactosidases also varies. Cortisone increases the activity of the neutral β -galactosidase in the jejunum and the ileum in the early suckling period. In older animals the response to cortisone is restricted to its effect on the ileum. The activity of the acid β -galactosidase is not affected by cortisone in the very young animals, but as the animals approach weaning the activity of the enzyme is depressed to a greater extent by cortisone.

Thus the effect of cortisone on the activity of acid β -galactosidase in the small intestine is different from its effect on the activity of other intestinal enzymes: whereas cortisone precociously increases the activity of invertase (Doell & Kretchmer, 1964), maltase (P. Sunshine, unpublished work) and duodenal alkaline phosphatase (Moog, 1953), it decreases the activity of the acid β -galactosidase. However, cortisone could be considered to have a maturation effect on the acid β -galactosidase, since it changes the activity of the enzyme towards

values found in mature animals. Cortisone has the opposite effect of adrenalectomy, which if performed in the weaning period delays the acquisition of adult activity values of invertase (Koldovský, Heringová & Jirsová, 1965a), acid β -galactosidase (Koldovský & Chytil, 1965), β -glucuronidase (Heringová *et al.* 1965) and sulphatase (Heringová, Koldovský, Yaffee & Jirsová, 1968).

This work was supported by Grants HD 02147, HD 391 and HD 49 from the U.S. Public Health Service (National Institutes of Health) and from the National Science Foundation. We thank Mrs Helen Jeavons for her excellent technical assistance.

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