Dietary-induced increase in lactase activity and in immunoreactive lactase in adult rat jejunum

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Adult rats that had been fed on a low-starch high-fat diet for 7 days were force-fed with either the same diet or isoenergetic diets containing 40% of energy as either sucrose or lactose. Within 12 h, the increase in jejunal lactase activity in sucrose- and lactose-fed rats was accompanied by a corresponding increase in immunoreactive lactase protein.

Activity of lactase in adult rat jejunum responds to increased intake of various carbohydrates, including starch and sucrose (McCarthy *et al.*, 1980; Bustamante *et al.*, 1981; Yamada *et al.*, 1981a,b), with a rapid increase. The possibility that such increases are due to increases in the amount of lactase protein was investigated in the present paper. Changes in amounts of lactase protein accompanying increases of lactase activity were determined by immunological methods in jejunum of rats fed on sucrose or lactose diets.

Materials and methods

Animals

Female Sprague–Dawley rats born in our animal colony were weaned at 30 days of age and fed on standard laboratory chow (Lab Blox; Allied Mills, Chicago, IL, U.S.A.) until 60 days of age. At that time they were fed on a synthetic low-starch (5% of energy) high-fat (73% of energy) diet for 7 days (Yamada *et al.*, 1981a). To control the food intake, they were then force-fed (via a plastic tube) with the low-starch diet or an isoenergetic sucrose diet or an isoenergetic lactose diet [40% of energy as sucrose or lactose, 37% of energy as fat and 22% of energy as protein, diluted with distilled water to make 9.9 kJ/ml (2.36 kcal/ml)]. Since decreased food intake or starvation can influence the specific activity of lactase (Ecknauer, 1978; Yamada *et al.*, 1983), we considered it important to control the food intake by force-feeding. Force-feeding was performed three times in 12 h: at 22:00, 02:00 and 06:00 h. The amount of diet was 6 ml per feeding, providing 88 kJ (21 kcal)/100 g body wt. per 12 h,

which corresponds to two-thirds of daily energy intake for rats of this age (Hahn & Koldovsky, 1967). This was considered adequate, because during the night hours rats in our animal colony consume about two-thirds of their daily intake. Rats had free access to water. The rats were killed by decapitation at 10:00 h, i.e. 12 h after the initial feeding of the sucrose and lactose diets.

Preparation of intestinal samples

The entire intestine was removed. The duodenum was discarded, and the jejunum was divided into three equal parts along its length. The proximal third of the jejunum (upper jejunum) was flushed with ice-cold saline (0.9% NaCl), and the mucosa was scraped from the remainder with a glass slide. Jejunal mucosa was homogenized with 4 vol. of 10 mM-potassium phosphate buffer (pH 7.0). A sample was taken for the determination of lactase activity in total homogenate: 0.6 ml of jejunal-mucosa homogenate (initial homogenate) was incubated at 37°C for 45 min with 0.288 mg of papain (Sigma, type I) and 0.72 mg of cysteine hydrochloride. This sample was designated as papain-treated homogenate and was then dialysed overnight against 10 mM-potassium phosphate buffer (pH 7.0) and centrifuged at 105 000 g for 60 min at 4°C. The supernatant was used for the determination of lactase activity and the quantification of immunoreactive lactase.

Preparation of antiserum

Lactase was purified from the proximal half of the jejunum of 14-day-old suckling rats essentially by the method of Tsuboi *et al.* (1979). Purification of lactase consisted of a sequence of steps including solubilization by papain, precipita-

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tion with $(\text{NH}_4)_2\text{SO}_4$ (35–55% saturation), Bio-Gel A-0.5m, hydroxyapatite, DEAE-Sephadex A-25 and Ultrogel AcA 34 column chromatography. This resulted in a 274-fold purification to a specific activity of $1034 \mu\text{mol/h}$ per mg of protein. Homogeneity of the purified enzyme was monitored by polyacrylamide-disc-gel electrophoresis. Antiserum was prepared by injecting rabbits subcutaneously on the back with $90 \mu\text{g}$ of purified lactase protein mixed with 0.5 ml of Freund's complete adjuvant. The injection was repeated after 2 weeks with $84 \mu\text{g}$ of lactase mixed with 0.5 ml of Freund's incomplete adjuvant, and 3 weeks later the rabbit was bled through the ear artery. Monospecificity of antiserum against lactase was confirmed by crossed immunoelectrophoresis against a papain-solubilized supernatant of small-intestinal homogenate containing lactase activity of $0.12 \mu\text{mol/h}$, sucrose activity of $0.41 \mu\text{mol/h}$ and $55 \mu\text{g}$ of protein in $12 \mu\text{l}$ applied. This loading of antigen produced a single precipitate peak that stained both with Coomassie Brilliant Blue and with the enzyme-staining reagent containing 100 mM -lactose, 4 units of glucose oxidase (Sigma, type V)/ml, 940 units of horseradish peroxidase (Sigma, type II)/ml and 0.3 mg of 3,3'-diaminobenzidine (Sigma, grade II)/ml in 50 mM -potassium phosphate buffer (pH 7.0).

Enzyme and immunological assays

Lactase activity was assayed as described by Koldovský *et al.* (1969). Protein was determined by the method of Lowry *et al.* (1951). Immunoreactive lactase was quantified by electroimmunoassay (rocket technique; Laurell, 1972). Purified lactase

($10 \mu\text{l}$) was applied as standard to consecutive wells in a concentration series of 5, 10, 30 and $50 \mu\text{g/ml}$. Samples ($10 \mu\text{l}$) of papain supernatant from animals on different diets were applied to the remaining wells. Electrophoresis was run at 13 mA for 20 h at 4°C . After electrophoresis, the glass plate was pressed and dried and stained with Coomassie Brilliant Blue. The plate was decolorized with 9% (v/v) acetic acid in 50% (v/v) methanol. The heights of the peaks were measured. Concentrations of immunoreactive lactase in experimental samples were extrapolated from the standard curve, which was linear between 10 and $50 \mu\text{g/ml}$.

Results and discussion

Results are summarized in Table 1. Body weight, food intake and total protein per jejunal segment were the same in all four groups studied.

Activity of lactase in homogenates prepared from sucrose- and lactose-fed animals was higher than in homogenates from control groups. This result confirms our previous report, which showed that the difference between control and sucrose-fed groups was highly significant (Yamada *et al.*, 1981b). Papain treatment of the homogenate resulted in a similar loss of lactase activity in all four groups (22–29%). The percentage of lactase activity solubilized in supernatants was the same for all dietary groups (Table 1).

Immunoreactive lactase protein was determined by electroimmunoassay as described in the Materi-

Table 1. Effect of feeding of sucrose diet and lactose diet on lactase activity and immunoreactive lactase in jejunum of adult rats. Results are means $\pm$ S.E.M. for four animals in each group. For details see the Materials and methods section.

^{a,b,c} The values not sharing a common superscript in the same row are significantly different from each other by one-way analysis of variance (ANOVA) ($P < 0.05$).

	Low-carbohydrate		Sucrose Force-fed	Lactose Force-fed
	<i>Ad lib</i>	Force-fed		
Body wt. (g)	210 ± 12	203 ± 16	203 ± 15	203 ± 14
Food intake (kJ/100 g body wt. per 12 h)	79.9 ± 1.3	88.7 ± 6.3	89.1 ± 2.7	89.1 ± 5.9
Jejunal protein (mg)	126 ± 6	121 ± 1	121 ± 2	113 ± 3
Lactase activity in jejunal homogenate ($\mu\text{mol/h}$ per mg of protein)	0.39 ± 0.05^a	0.42 ± 0.03^a	0.83 ± 0.06^b	$0.63 \pm 0.08^{a,b}$
Lactase activity recovered in papain extract				
As % of that in initial homogenate	67 ± 7	69 ± 4	63 ± 3	69 ± 6
As % of that in papain-treated homogenate	86 ± 2	88 ± 1	90 ± 1	90 ± 0.2
Lactase activity (LA) in papain extract ($\mu\text{mol/h}$ per mg of protein)	1.21 ± 0.15^a	$1.27 \pm 0.14^{a,b}$	2.70 ± 0.20^c	$1.85 \pm 0.22^{b,c}$
Immunoreactive lactase (IRL) ($\mu\text{g/mg}$ of protein)	1.97 ± 0.32^a	2.10 ± 0.28^a	3.80 ± 0.19^b	$2.97 \pm 0.31^{a,b}$
LA/IRL ($\mu\text{mol/h}$ per μg)	0.63 ± 0.04	0.61 ± 0.03	0.71 ± 0.02	0.62 ± 0.02

als and methods section. Similar results to those for determination of lactase activity were obtained. The amount of immunoreactive lactase in papain-solubilized supernatants was significantly increased within 12 h in sucrose-fed rats; in those fed with lactose a similar trend (50% increase) was observed. The ratio of lactase activity to immunoreactive lactase, which represents the lactase activity per unit weight of enzyme protein, was practically the same between groups.

Therefore we conclude that the dietary-induced rapid increase in lactase activity occurs concomitantly with increases in immunoreactive lactase protein. The mechanisms leading to the increase of the amount of lactase protein, whether due to changes of the rate of synthesis or degradation of lactase, have to be determined. Similarly the more pronounced effect of the sucrose diet than of the lactose diet is another open question resulting from these studies.

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