DIETARY-INDUCED RAPID INCREASE OF RAT JEJUNAL SUCRASE AND LACTASE ACTIVITY IN ALL REGIONS OF THE VILLUS

K. YAMADA, S. BUSTAMANTE and O. KOLDOVSKÝ*
Departments of Pediatrics and Physiology, University of Arizona Health Sciences Center, Tucson, AZ 85724, USA

Received 5 May 1981

1. Introduction

Increased intake of sucrose leads to an increase of intestinal sucrase activity in rats [1,2] and human subjects [3]. Rats fed purified diets with high amounts (71 cal%) of starch or sucrose (diets containing only α-glucoside linkages) exhibited significantly higher activity of intestinal sucrase as well as lactase compared to those rats fed isocaloric diets with a low (6 cal%) carbohydrate content [4–7].

Since the work in [8] it has been recognized that the enterocytes mature both morphologically and biochemically while migrating from the crypts to the villus. Activity of sucrase and lactase is absent in the crypt cells and exhibits an increase in enterocytes as they migrate towards the top of the villus [9,10]. This led to a logical question: at which level of the villus (i.e., at which period of their life span) are the enterocytes capable of responding to a dietary change with an alteration of disaccharidase activity. This question was explored [11,12]: in adult rats where the sucrase activity was lowered by starvation, refeeding with sucrose led to an increase of sucrase activity only in the cells of the crypt, and the activity increased as the enterocytes moved towards the villus top.

Similar questions regarding the lactase activity have not been studied yet most probably because:

(i) In the adult rat, starvation for 2–3 days leads to a decrease of sucrase activity, but not to a decrease in the lactase activity [11,13] (unpublished);

(ii) The carbohydrate effect on lactase, until recently, was questionable [6,14].

Since the dependency of lactase activity on dietary carbohydrate content in adult rats was demonstrated [4–7], we decided to explore the locus of the effect of dietary change on intestinal lactase activity. In our experiments, the activity of sucrase and lactase was lowered not by starvation with sucrase [11,12], but by feeding the rats a low carbohydrate diet. Here we show that both lactase and sucrase activity were rapidly increased within 18–24 h in rats fed the high sucrose diet in all regions of the villus.

2. Materials and methods

Female Sprague-Dawley rats, bred in our own animal colony were weaned at 30 days of age, and fed until 88 days of age a standard laboratory chow (Lab-Blox, Allied Mills, Chicago). After 88 days of age they were fed a synthetic, low starch diet (5 cal% starch, 73 cal% fat, 21 cal% protein) for 14 days. The animals were then either continued on the same diet or started on an isocaloric 'high' sucrose diet (70 cal% sucrose, 7% fat, 21% protein; for detailed composition of the diets see [7]) at 3 p.m. or 9 a.m. for 18 h or 24 h, respectively. All rats were fed ad libitum and had unrestricted access to water. Food consumption was measured by weighing the food every morning. Fresh diet was given every second day. Rats were sacrificed by decapitation at 9–10 a.m., in a fed stage. The entire small intestine was removed, duodenum discarded, and the jejunoileum was divided into 3 equal parts along its length. It was then flushed with cold saline solution and frozen at −20°C before the assays of disaccharidases and protein were performed.

To assay the activity of disaccharidases at different levels of the villus, a 5 × 5 mm segment of jejunum was sectioned within a cryostat at −18°C as in [9,10]. Horizontal sections were cut 10 μm thick. At various

* To whom correspondence should be addressed

depths into the villus–crypt unit, a section was mounted on a microscope slide for immediate inspection of histology under a phase-contrast microscope. The tissue blocks were sectioned through the submucosa into the muscular layer. Every 10 consecutive sections were combined and homogenized in 0.5 cm^3 distilled water by vortex shaking. Assays of lactase and sucrase activities were performed on each homogenate as in [15]. The lactase assay mixture contained *p*-chloromercuriobenzoate (PCMB), supplied by the Aldrich Chemical Co. (Milwaukee WI), to inhibit any residual lysosomal acid β-galactosidase activity [16]. All enzyme determinations were made under condition of linear activity with time and concentration of the enzyme. Protein was determined according to [17]. Enzyme activity was expressed as μmol liberated glucose . mg $tissue^{-1}$ protein (spec. act.) . h^{-1}.

When cell migration was studied, the technique in [11] was used. At the same time as the initiation of sucrose feeding, animals received 100 μCi [*methyl*-^{3}H]-thymidine intraperitoneally. From an ~10 × 10 mm segment of jejunum (see above), 10 slices of 10 μm thickness/tube were prepared using cryostat sectioning. These were solubilized in Protosol (New England Nuclear, Boston MA) to which a standard toluene–PPO–POPOP mixture (Liquifluor, New England Nuclear) was added. Samples were counted in a Beckman liquid scintillation spectrometer (LS-230). With this technique similar data as that in [11] were obtained (see section 3).

All chemicals used were of reagent grade. Means of different groups were compared by a one-way analysis of variance [18]. The level of significance chosen was $p < 0.02$.

Table 1
Effects of feeding a high sucrose diet for 18 or 24 h

	High fat low starch (18 h)	Low fat high sucrose (18 h)		High fat low starch (24 h)	Low fat high sucrose (24 h)	
Numbers of animals	5	p[a]	4	5	p[a]	5
Body weight (g)	265 ± 12	n.s.	276 ± 13	255 ± 9	n.s.	248 ± 11
Food intake (cal/100 g body wt/18 or 24 h)	18.8 ± 2.0	n.s.	19.2 ± 1.2	23.5 ± 2.5	n.s.	17.0 ± 1.6
Height of total intestinal wall (mm)[b]	0.89 ± 0.06	n.s.	0.95 ± 0.05	1.09 ± 0.05	n.s.	1.06 ± 0.04
Leading edge of the radioactivity (% of total intestinal wall)	54 ± 1	n.s.	53 ± 4	69 ± 2	n.s.	65 ± 3
Intestinal protein (mg/segment)						
proximal segment	202 ± 15	n.s.	227 ± 19	186 ± 13	n.s.	174 ± 12
middle segment	199 ± 10	n.s.	210 ± 8	207 ± 13	n.s.	188 ± 11
distal segment	120 ± 3	n.s.	128 ± 6	152 ± 11	n.s.	145 ± 10
Lactase (μmol . mg $protein^{-1}$. h^{-1})						
proximal segment	0.25 ± 0.04	0.001	0.85 ± 0.04	0.44 ± 0.05	0.001	1.07 ± 0.08
middle segment	0.52 ± 0.06	0.001	1.00 ± 0.03	0.68 ± 0.06	0.01	1.22 ± 0.06
distal segment	0.22 ± 0.03	0.005	0.34 ± 0.02	0.19 ± 0.04	0.01	0.31 ± 0.05
Sucrase (μmol . mg $protein^{-1}$. h^{-1})						
proximal segment	0.69 ± 0.11	0.001	2.29 ± 0.16	0.66 ± 0.13	0.001	2.58 ± 0.31
middle segment	0.40 ± 0.13	0.001	1.41 ± 0.08	0.37 ± 0.07	0.001	1.38 ± 0.23
distal segment	0.35 ± 0.05	0.005	0.74 ± 0.05	0.28 ± 0.07	0.001	0.65 ± 0.07
Maltase (μmol . mg $protein^{-1}$. h^{-1})						
proximal segment	6.9 ± 0.4	0.001	15.0 ± 0.6	9.3 ± 1.2	0.001	18.7 ± 1.0
middle segment	9.0 ± 0.8	0.005	14.0 ± 0.7	9.5 ± 0.4	0.001	15.9 ± 0.4
distal segment	7.9 ± 0.3	0.01	9.9 ± 0.5	6.3 ± 0.8	0.05	9.3 ± 0.8

[a] *p*-Value (experimental *vs* control); n.s., not significant
[b] Determined by multiplying 10 μm (height of the individual slice) × number of slices

The time elapsed between the '18 h' and '24 h' experiments was 3 months; Values are given as means ± SEM

3. Results and discussion

Experiments were performed on two different groups. In the first group, the changes of disaccharidase activity was examined 18 h after the introduction of the high sucrose diet. In the second group which consisted of animals also fed this diet (for 24 h), corresponding controls (rats consuming low starch diet) were sacrificed at the same time as experimental animals.

Food intake, body weight, protein content, height of the villus–crypt columns were practically the same in all groups of rats. Rate of cell migration did not differ between the experimental and corresponding control group. The 24 h migration rate values were significantly higher than the 18 h values ($p < 0.01$) (table 1).

Feeding the rats the high fat low starch (5 cal%) purified diet for 14 days led to a decrease of activity of lactase and sucrase in the intestinal homogenate. This was in good agreement with the results in [4–7]. Changing the diet from the low starch (5 cal%) diet to the high sucrose (70%) diet led to a significant increase of lactase and sucrase activity in all three intestinal segments within 18 h and 24 h.

The effect of the diets on the distribution of lactase and sucrase activity along the height of the villus–crypt columns is shown in fig.1. As in [10,19], the activities of disaccharidases in serial homogenates were related to an 'idealized villus–crypt unit'. This maneuvre allowed data from a number of animals to be easily compared.

In agreement with the determination of disaccharidases in whole jejunal homogenate, their activity in serial sections was higher in rats fed the high sucrose diet that in rats fed the low starch diet. There were significant increases in all regions of the villus–crypt columns. Furthermore, there was also a significant increase in sucrase activity not only in the lower villus but also in the villus tip. Lactase activity also increased equally in all regions.

Comparison of the distribution pattern of disaccharidases activities along the height of the villus–crypt column with the determination of the rate of cell migration revealed that both lactase and sucrase activities increased not only in the immature enterocytes but also in enterocytes that have already left the crypt at the time of the initiation of the dietary change. This is demonstrated in the case of lactase using the following example. Cohorts of enterocytes in control animals at the 40% level, (i.e., already on the lower portion of the villus), exhibited lactase activity of 0.09 ± 0.02 μmol . mg protein^{-1} . h^{-1}. These migrated within the following 18 h up to 80% position; their activity was 0.30 ± 0.06. On the other hand, in rats fed the high sucrose diet, the activity at a corresponding villus height was 0.92 ± 0.09. This difference (depicted in fig.1) is significant ($p < 0.01$). Similar phenomenon existed in the case of sucrase activity also.

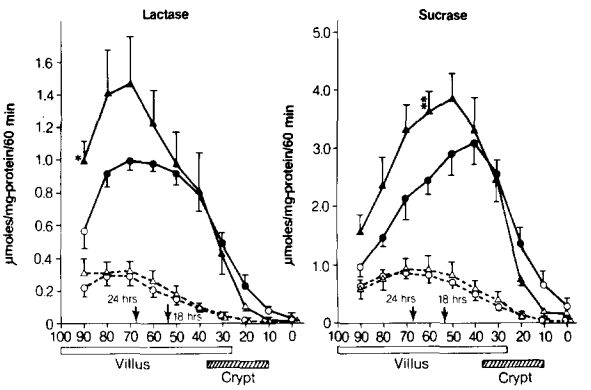

Fig.1. Effects of high sucrose diet feeding for 18 h or 24 h on jejunal lactase and sucrase specific activities along the villus–crypt columns. Abscissa depicts total height of the intestinal wall, with 100% representing the top part of the villus and 0% the bottom of serosal side. Villus and crypt portions are depicted with rectangles; the overlapping area is the crypt–villus transition (mix) zone. Mean and SEM are given; N of individual animals are as in table 1. The dotted line represents control groups fed high fat low starch diet ((○) 18 h; (△) 24 h) and solid line represents experimental groups fed low fat high sucrose diet ((●) 18 h; (▲) 24 h). Full symbol depicts significantly different values from corresponding control of the same location; **significant difference ($p < 0.02$) between the values of the group fed sucrose for 18 and 24 h, *same at $p < 0.05$ level. The arrows indicate the leading edge of thymidine labeled cells as in section 2.

Further studies are needed to explore the mechanisms involved in the dietary adaptation of sucrase and lactase activity. Our data allow us to conclude that in adult rats, activity of the disaccharidases can be modified in mature enterocytes as well as in the immature enterocytes. Until this study, it was believed that only immature (crypt) enterocytes are competent to react to initiation of sucrose feeding with an increase of activity of sucrase. At present we can only speculate reasons for the discrepancy between data obtained in animals fed high fat low starch diet, and rats that have first been starved and the refed.

Acknowledgements

This study was supported by NIH research grant AM 27624. The excellent technical assistance of Robert Lindberg is appreciated.

References

[1] Blair, D. G. R., Yakimets, W. and Tuba, J. (1963) Can. J. Biochem. Physiol. 41, 917–929.
[2] Deren, J. J., Broitman, S. A. and Zamcheck, N. (1967) J. Clin. Invest. 46, 186–195.
[3] Rosenweig, N. S. and Herman, R. H. (1968) J. Clin. Invest. 47, 2253–2263.
[4] Koldovský, O., Gasparo, M., Lau, H., Brown, S., Coates, P. and Sonawane, B. (1980) Fed. Proc. FASEB 39, 500.
[5] McCarthy, D. M., Nicholson, J. A. and Kim, Y. S. (1980) Am. J. Physiol. 239, 6445–6451.
[6] Bustamante, S., Gasparo, M., Kendall, K., Coates, P., Brown, S., Sonawane, B. and Koldovský, O. (1981) J. Nutr. 111, in press.
[7] Yamada, K., Bustamante, S. and Koldovský, O. (1981) Biochim. Biophys. Acta (in press).
[8] Leblond, C. P. and Stevens, C. E. (1948) Anat. Rec. 100, 357–378.
[9] Nordström, C., Dahlqvist, A. and Joseffson, L. (1968) J. Histochem. Cytochem. 15, 713–721.
[10] Boyle, J. T., Celano, P. and Koldovský, O. (1980) Gastroenterol. 79, 503–507.
[11] Ulshen, H. M. and Grand, R. J. (1979) J. Clin. Invest. 64, 1097–1102.
[12] Raul, F., Simon, P. M., Kedinger, M., Grenier, J. F. and Haffen, K. (1980) Biochim. Biophys. Acta 630, 1–9.
[13] McNeill, L. K. and Hamilton, J. R. (1971) Pediatrics 47, 65–72.
[14] Koldovský, O. (1981) in: Carbohydrate Metabolism and Its Disorders (Whelan, W. J. ed) vol. 3, Academic Press, New York, in press.
[15] Dahlqvist, A. (1964) Anal. Biochem. 7, 18–25.
[16] Koldovský, O., Asp. N. G. and Dahlqvist, A. (1969) Anal. Biochem. 27, 409–418.
[17] Lowry, C. H., Rosebrough, W. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 263–275.
[18] Sokal, R. R. and Rohlf, F. J. (1969) in: Biometry, pp. 175–253, Freeman, San Francisco.
[19] Herbst, J. J. and Koldovský, O. (1972) Biochem. J. 126, 471–476.