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Effect of hydrocortisone on sialyltransferase activity in the rat small intestine during maturation

Changes along the villus-crypt axis and in fetal organ culture

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Sialyltransferase activity was assayed in rat intestinal cells isolated as fractions reflecting the villus-crypt axis of differentiation. In 13-day-old rats both endo- and exogenous sialyltransferase activity reached their maximum in undifferentiated crypt cells and their peaks overlapped. In contrast, sialyltransferase of the adult intestine was 4-fold lower than that of sucklings in the crypts, with slight tendency to be transferred to the villus cells. Hydrocortisone applied to 10-day-old rats caused three days later a precocious drop of sialyltransferase activity in the crypt cells. Unlike in vivo, glucocorticoid responsiveness was accompanied by increased sialyltransferase activity in fetal small intestine cultivated for 17 days.

Sialyltransferase; Hydrocortisone; Villus-crypt axis; Fetal organ culture

1. INTRODUCTION

Developmental studies suggest that during transition from suckling to weaning the highly sialylated forms of jejunal brush-border enzymes of suckling rats are converted to asialylated (adult) forms [1]. Administration of hydrocortisone to suckling rats speeds up this process [2] and evokes the appearance of asialylated forms first in the crypt cells [3]. Evidence for the higher sensitivity of undifferentiated crypt cells to hydrocortisone is provided by the fact that more basic p*I* values of less sialylated sucrase-isomaltase (EC 3.2.1.48-10), glucoamylase (EC 3.2.1.20) and dipeptidylpeptidase IV (EC 3.4.14.5) are induced in the crypts prior to their appearance at the villus tips. To explain this phenomenon we studied the effect of hydrocortisone on sialyltransferase activity in fractions of sequentially isolated cells proceeding from the tip of the villus to the base of the crypt. We observed that the significant drop in total mucosal sialyltransferase system in response to cortisone found previously [4,5] is confined mostly to undifferentiated cells [6]. Sialyltransferase was tested also in cultured fetal intestine where no asialylated forms of the brush-border enzymes were observed in the presence of dexamethasone in the culture medium [7].

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2. EXPERIMENTAL

2.1. *Materials*

A litter of 10-day-old rats was given a single s.c. injection of 1.5 mg/20 g body wt of hydrocortisone in 0.154 M NaCl and a litter of 10-day-old control rats were given the same volume of saline. On day 13 intestinal jejunum of both control and hydrocortisone-treated rats was used for intestinal cell isolation. Similarly, jejunum of control adult rats (92-day-old) served as a source of isolated cells.

Explants of small intestinal tissue from 17-day-old fetuses of pregnant Wistar rats were cultured for 17 days at 37°C in a humidified atmosphere with 5% CO_2 in air.

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2.2. *Methods*

Intestinal cells were isolated from everted segments of the jejunum essentially according to [8] in 14 fractions detached sequentially from the tip of the villus to the base of the crypt in the course of 90 min.

Fetal organ cultures were incubated in Eagle's minimal essential medium with 2 mM glutamine, 1 mM Na-pyruvate, 10 mM Hepes, 40 mg/ml gentamycin and 5% heat-inactivated fetal calf serum. Dexamethasone, a stable synthetic analogue of hydrocortisone, was used at a concentration of 80 nM. Half of the medium volume was replaced every two days.

Jejunal cell fractions and fetal organ cultures were washed twice with phosphate-buffered saline and the 900× *g* pellet was homogenized in 10 vols of 50 mM Tris-HCl at pH 7.4. The homogenate was centrifuged at 100000× *g* for 60 min and the pellet resuspended in 50 mM Tris-HCl (pH 7.4) and used for protein [9] and sialyltransferase [10] assays. Sialyltransferase assays were carried out with 100 μl aliquots containing 200–500 μg protein of the jejunal cell preparation or 100 μg protein of fetal organ culture preparation in a medium with additions of 20 μl of 0.2 M Tris-HCl (pH 6.5), 5 μl of 0.2 M $MnCl_2$, 5 μl of 0.1% Triton X-100, 20 μl asialofetuin (10 mg/ml of 0.2 M Tris-HCl at pH 6.5) and 5 μl [^{14}C]CMP-NeuAc (Amersham, International; 40000 cpm). The assay samples were incubated at 37 °C for 60 min. Exogenous enzyme activity was calculated as the difference between the total activity with asialofetuin present and the activity with only endogenous intestinal cell acceptors.

The percentage of isolated cells was based on the cumulative protein content in 100000× *g* pellets of sequentially isolated cells fractions.

3. RESULTS AND DISCUSSION

Fig.1 shows the villus-crypt gradient of endogenous and exogenous jejunal sialyltransferase activity of the adult rat. It appears that villus cells transfer sialic acid from CMP-sialic acid slightly better to both endogenous acceptors and asialofetuin. Previous reports by two groups were different with respect to the localization of sialyltransferase activity in the crypt area of the small intestine from adult rat. Like in our studies, Weiser [11] has shown a higher endogenous activity of sialyltransferase in the villus cells but with a more pronounced difference between the highest level of this enzyme in the villus area and the lowest level in the crypt cells. This apparent discrepancy might be explained by the fact that our examination of the membranous fraction (100000 × *g* pellet) revealed sialyltransferase also in Golgi apparatus membranes, while the intact cell preparation used [11] covers mostly the post-Golgi localization: the surface sialyl-transferase activity. A distinctly different distribution of total cellular

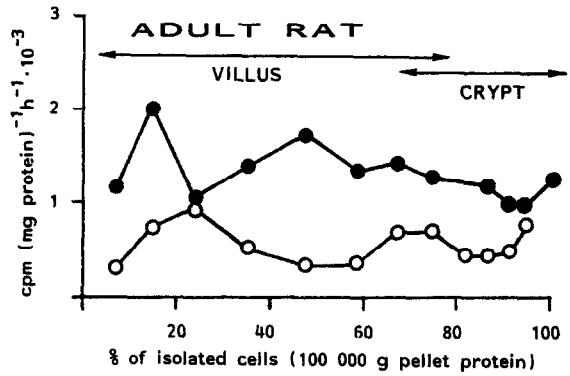

Fig.1. Gradient of sialyltransferase activity along the villus-crypt axis. Endogenous activity (○), exogenous activity (●). The results were obtained from jejunums of three 92-day-old rats.

sialyltransferase was observed by Kim et al. [12] who used the planar sectioning method and the same exogenous acceptor as in our studies. They showed a considerably higher activity of sialyltransferase in the crypt cells than in the villus cells. At present it is difficult to establish why our findings on the sialyltransferase level in the crypt cells are in disagreement with the results of Kim et al. [12]. The possibility may be considered that during preparation of isolated cells by the 'washing' technique some specific shedding of sialyltransferase into the incubation medium takes place as described for the small intestine by Ratnam et al. [13]. However, it does not seem to be the case (see below the data for the suckling intestine). On the other hand, the planar sectioning method does not control for the possible contribution of other cell types besides enterocytes to sialyltransferase activity, above all in the crypt area. The nearly two-fold higher sialic acid content of the villus tip cells as compared with the crypt cells found in adult rat intestine by Gupta et al. [14] corresponds much better to the distribution of sialyltransferase activity given in fig.1.

Data in fig.2 (upper panel) show that in 13-day-old rats the endo- and exogenous activity of sialyltransferase reach maximum in the crypt region. At this location it differs most from the adults, being about 4-fold higher. These data suggest that the developmental decrease of overall mucosal sialyltransferase activity described by Chu and Walker [5] originates in the crypts and/or in the area of villus-crypt junction. Changes in the

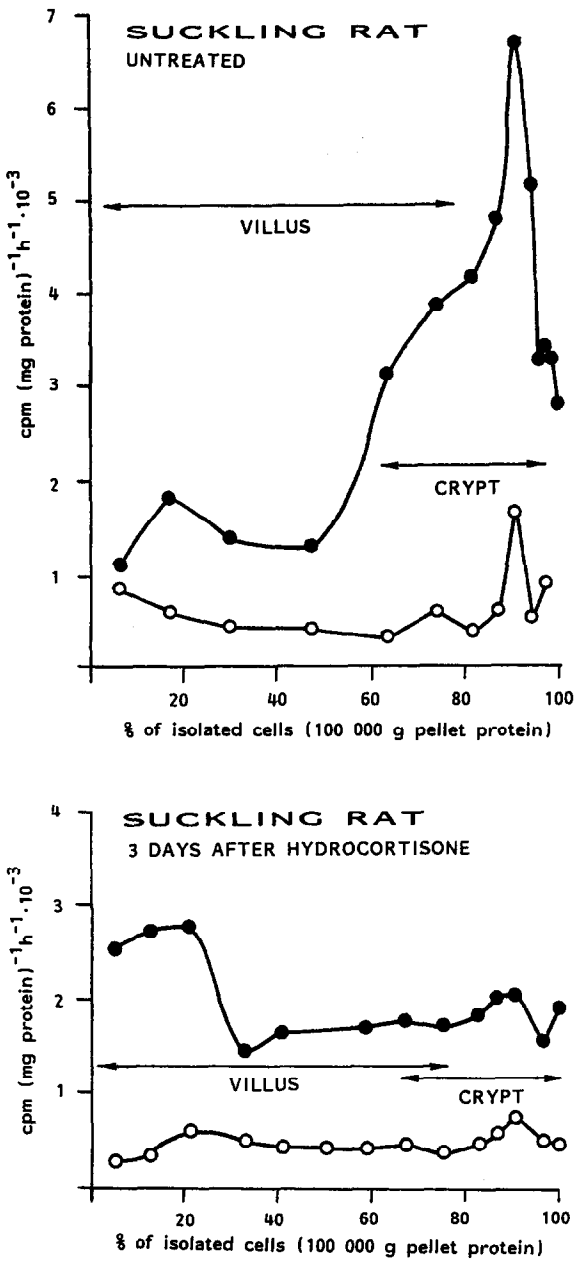

Fig.2. Gradient of sialyltransferase activity along the villus-crypt axis. (Upper panel) 13-day-old rat and (lower panel) 13-day-old rat three days after hydrocortisone administration. Endogenous activity (○), exogenous activity (●). Results for both panels were obtained from 10 jejunums each.

distribution of glycosidically bound sialic acid along the villus-crypt axis with increasing age [15] also match well with our results.

Fig.2 (lower panel) depicts the distribution of sialyltransferase activity three days after the application of hydrocortisone to 10-day-old rats. Hydrocortisone brings about a decrease of exo- and endogenous sialyltransferase activity in the crypt cells by 71% and 58%, respectively. This drop in sialyltransferase activity in the crypt region is entirely consistent with the formation of a gradient of p*I* values of brush-border hydrolases along the villus-crypt axis due to the precocious appearance of their asialylated forms [3] and with precocious decrease of total sialic acid [15] in the crypts under the effect of hydrocortisone. These dynamic changes include stimulation of brush-border glucoamylase (not shown) and induction of sucrase-isomaltase which start also in the undifferentiated enterocytes [16]. Coincidence of a rapid enrichment by newly appearing membrane hydrolases with local lack of sialyltransferases results in a decreased sialylation in the crypt cells prior to villus cells.

Table 1 demonstrates an increase of about 1.7-fold in sialyltransferase activity of fetal cultures when 80 nM dexamethasone was present in the culture medium. In accordance with this finding is the persistently high degree of sialylation of dipeptidylpeptidase IV and no decrease in the sialylation of the de novo synthesized glucoamylase in fetal cultures in response to dexamethasone [7].

Corticoids thus appear to act in a different manner on sialylation in vivo and in vitro; perhaps, the secreted sialyltransferase [13,17] plays its catalytic role better in vitro. The corresponding effect of dexamethasone on cultured hepatocytes has been explained as primarily involving the sialyltransferase pool destined for secretion [18].

The process of synthesis of the brush-border en-

Table 1

Sialyltransferase activity in cultured fetal intestine

	cpm per mg protein per h
Control	1725
80 nM dexamethasone	2948

Each value was obtained from fetal cultures grown in 4 culture dishes. There were segments of 5 fetal small intestines per culture dish. Sialyltransferase is presented as exogenous activity expressed in counts per min per mg protein of the 100 000 × *g* pellet per h.

zyme proteins and that of the modulation of their glycosylation may not be chronologically related during ontogeny. Incorporation studies of radioactively labeled sugars indto microvillar membranes [19] support this view.

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