Effect of hydrocortisone on the desialylation of intestinal brush-border enzymes of the rat during postnatal development

Jiří Kraml, Jiřina Kolínská+, Libuše Kadlecová, Marie Zákostelecká+ and Zdeněk Lojda*

*1st Department of Medical Chemistry, and *Laboratory of Histochemistry, Faculty of Medicine, Charles University, Kateřinská 32, 121 08 Prague 2* and +*Institute of Microbiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czechoslovakia*

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Hydrocortisone acetate or hemisuccinate (75 mg/kg body mass) applied to rats i.m. and/or s.c. on the 9th and 10th postnatal days causes a precocious decrease of sialic acid content of the small intestinal brush-border membrane. On the 15th postnatal day the bound sialic acid of the whole membrane fraction drops to almost half of the values of control animals and to one third of the control values for the papain-solubilized membrane proteins. The hydrocortisone effect is manifested on isoelectric focusing zymograms by a faster increase of p*I* of the solubilized brush-border enzymes on the 12th and 15th postnatal days.

Brush-border enzyme *Sialic acid* *pI change* *Hydrocortisone* *Postnatal development* *Rat small intestine*

1. INTRODUCTION

The papain-solubilized intestinal brush-border enzymes of infant rats exhibit an increase in *pI* values till the age of 20–30 days. Analogous changes can be observed on treating solubilized brush-border fractions of young rats in vitro with neuraminidase [1], which brings about desialylation of the enzymes.

Hydrocortisone administration speeds up the process of redifferentiation of the intestinal mucosa during postnatal development [2]. Our results showing an increase of p*I* values of some brush-border enzymes under the effect of hydrocortisone [3] indicated a role of this hormone in the sialylation and desialylation process. Here we studied the relationship between the sialic acid content and p*I* values of intestinal brush-border enzymes after hydrocortisone administration.

Abbreviations: IEF, isoelectric focusing; Zwittergent 3-14, *N*-tetradecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate; 2-NNap, 2-naphthylamide; 2-NNapOMe, 4-methoxy-2-naphthylamide; 2-Nap, 2-naphthyl; FBB, Fast Blue B salt

2. MATERIALS AND METHODS

2.1. Hydrocortisone administration and isolation of brush-border membranes

A group of 5–8 suckling rats was given i.m. and/or s.c. injections of hydrocortisone acetate (Spofa, United Pharmaceutical Works, Prague) or hemisuccinate (Solu-Cortef, Upjohn, Puurs) on postnatal days 9 (50 mg/kg body mass) and 10 (25 mg/kg body mass). Another group was not given hydrocortisone and served as a control. Both control and injected rats were killed at days 12 or 15. 70-Day-old rats were used as a model for adult IEF zymograms of brush-border enzymes

Isolated brush-border membranes were prepared from mucosal scrapings of the jejunum essentially according to [4]. Mixed samples of brush borders from 5–8 animals each were resuspended in 0.005 M Na_2 EDTA (pH 7) and dialyzed overnight against 0.05 M KCl prior to solubilization.

2.2. Solubilization of brush-border proteins and effect of neuraminidase in vitro

The membranes were solubilized either by deter-

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gent or by proteolytic treatment. Zwittergent 3-14 (Calbiochem-Behring, La Jolla), a detergent suitable for electrofocusing of integral membrane proteins [5] was used at 1% concentration at 4°C for 60 min. The zwitterionic detergent was found to be a good solubilizer of brush-border enzymes [3], so that weak activities of α-glucosidases during the early postnatal development can be well demonstrated in IEF gels. Proteolysis with papain (Papaya latex, 2× crystallized, Koch-Light, Colnbrook) proceeded at 37°C for 60 min (33 μg/mg protein; activated with cysteine hydrochloride 3.3 μg/mg protein). After solubilization the 105 000 × g (4°C) supernatants were dialyzed against 0.01 M KCl before application to the IEF gel. The protein concentration was 1–3 g/l.

In some experiments the solubilized brush-border fractions were treated with neuraminidase (*Clostridium perfringens,* Boehringer, Mannheim) as in [1].

2.3. *Determination of sialic acid*

Sialic acid content in the whole brush borders or in the solubilized fractions was estimated after hydrolysis with 0.05 M H_2SO_4 at 80°C for 60 min as in [6]. *N*-Acetylneuraminic acid (Koch-Light) was used as a standard. Protein was determined according to [7], with bovine serum albumin as standard.

2.4. *Analytical isoelectric focusing and histochemical demonstration of enzymes in agarose gels [1]*

Thin-layer analytical IEF was performed in Agarose IEF (Pharmacia, Uppsala) in the pH interval 3–10 with IEF pI calibration kits (Pharmacia). Enzyme activities in the gel, i.e., α-glycosidases, dipeptidyl peptidase IV (EC 3.4.14.5) and γ-glutamyltransferase (EC 2.3.2.2), were demonstrated by azo-coupling reactions as in [1] and [8]. When detergent solubilization was used, 0.05% Zwittergent was added to the gel medium.

3. RESULTS AND DISCUSSION

In the group treated with hydrocortisone acetate on the 9th and 10th postnatal days the sialic acid bound in brush borders measured 3 days after hormone application was the same as in controls, while 6 days after hormone application it dropped from the control value of 93.5 ± 10.03 to 51.2 ± 3.47 μmol/kg membrane protein ($p < 0.01$) (fig.1). In the corresponding papain-solubilized brush-border fractions the differences were even larger, i.e., 115.7 ± 18.1 μmol/kg protein in controls and 37.33 ± 9.86 μmol/kg protein after the hormone treatment ($p < 0.01$).

There was a significant ($p < 0.01$) increase of bound sialic acid in the brush borders of 15-day-old control animals as compared with 9- and 12-day-old ones. A similar, temporary increase at this time period was also observed in [9]; however, the following desialylation process in the membrane continues up to 60 days after birth [9]. This was also confirmed by our previous findings of a general increase of pI values of intestinal brush-border enzymes of the rat during postnatal development [1].

Analytical IEF of solubilized brush-border enzymes confirmed the effect of hydrocortisone on the desialylation process in the postnatal period.

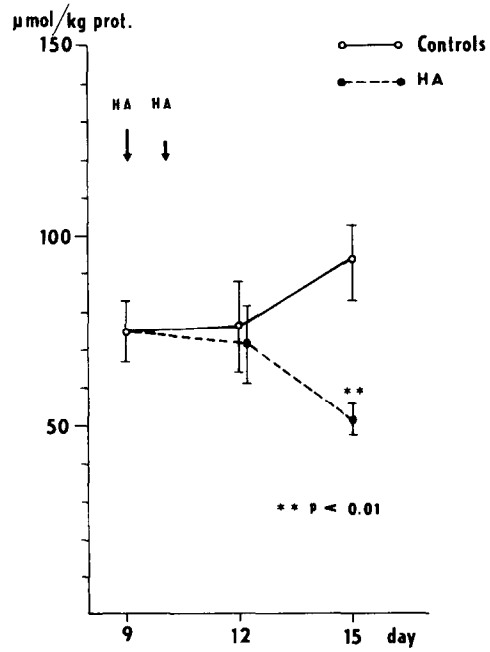

Fig.1. Sialic acid content of the rat intestinal brush-border membrane after hydrocortisone-acetate (HA) treatment (75 mg/kg body mass) (●--●) compared with controls (○—○). Each point is a mean of estimations from 3–5 brush-border preparations.

α-Glycosidases were chosen as a developmental marker, as it is known that the sucrase–isomaltase complex (EC 3.2.1.48-10) is absent in newborn rats and appears first at the beginning of the third week of life [10]. Precocious appearance of this enzyme complex can be induced after hydrocortisone administration [11,12]. The effect of hydrocortisone on IEF zymograms of α-glycosidases is seen in fig.2. The only α-glucosidase, the weakly active glucoamylase (EC 3.2.1.20), of 12-day-old rats is sialylated, forming a diffuse band in the range of pI 4.25–4.50. Thus, it differs from a sharp band (pI 4.55) of sialic acid-free glucoamylase of adult rat intestine (fig.2a). Neuraminidase treatment in vitro converts the acidic form of the glucoamylase of 12-day-old rats into the more basic (adult) form. Administration of hydrocortisone to 9-day-old rats promotes an elevation of glucoamylase and precocious appearance of sucrase–isomaltase which, 3 days later, results in an intense diffuse band, a mixture of both enzyme activities. Desialylation by neuraminidase produces the pattern of glucoamylase (pI 4.55) and sucrase–isomaltase (pI 4.8) bands found in adults [1] (fig.2a). It is evident that hydrocortisone causes the simultaneous appearance of both sialylated (more acidic) and desialylated forms (pI 3.85–4.75) of the two enzymes by the third day after hydrocortisone administration (fig.2b). In 15-day-old rats, 6 days after hydrocortisone injection there is already seen a differentiation of the two enzyme bands (pI 4.3–4.75) (fig.2c). It resembles the adult pattern produced after neuraminidase action.

The greatest increase in pI values of intestinal brush-border enzymes during the postnatal development of the rat was observed for dipeptidyl peptidase IV and γ-glutamyltransferase [1]. Zymograms of dipeptidyl peptidase IV are shown in fig.3. As demonstrated in [1], in baby rats the enzyme exhibits a large heterogeneity due to various degrees of sialylation. This is also apparent in the controls on days 12 and 15 for both modes of solubilization, with maxima of stained bands around pI 4.55. On day 12, 3 days after hydrocortisone application the bands are broader, displaying both sialylated and desialylated (more basic) forms. Later, on the 15th day, 6 days after hydrocortisone treatment, the forms with pI around 5.5 prevail. This pI value is typical for adult forms of the enzyme or for the desialylated forms obtained after

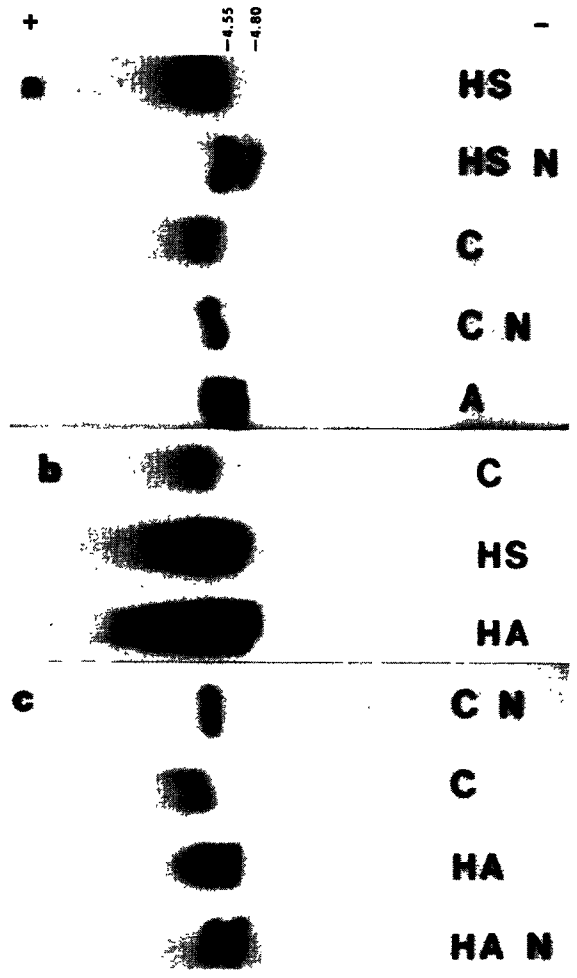

Fig.2. Effect of hydrocortisone on IEF zymograms of brush-border α-glycosidases. Pharmalyte 3–10; stained with 2-Nap-α-D-Glcp, FBB (pH 6.0). Brush borders were solubilized with Zwittergent 3-14. C, controls; N, treated with neuraminidase; HA, hydrocortisone acetate; HS, hydrocortisone hemisuccinate; A, adult rats. (a) 12-day-old and adult rats; HS (50 mg/kg body mass); (b) 12-day-old rats; HS or HA (75 mg/kg body mass); (c) 15-day-old rats; HA (75 mg/kg body mass). Numbers above indicate pI values of adult forms of glucoamylase (4.55) and sucrase–isomaltase (4.80).

neuraminidase treatment of preparations from younger rats [1]. Similar effects of hydrocortisone were also observed for γ-glutamyltransferase, which displays a larger heterogeneity than the former enzyme. Three days, and even more so 6 days after hydrocortisone application the bands are shifted from the range of pI 4.5–6.6 to the

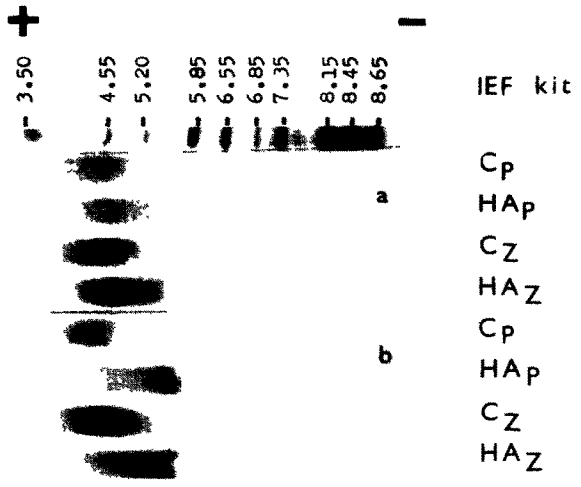

Fig.3. Effect of hydrocortisone on IEF zymograms of brush-border dipeptidyl peptidase IV. Pharmalyte (pH 3–10); stained with Gly-L-Pro-2-NNapOMe, FBB (pH 7.2). (a) 12-day-old rats; (b) 15-day-old rats. C, controls; HA, hydrocortisone acetate (75 mg/kg body mass); P, brush borders solubilized with papain; Z, brush borders solubilized with Zwittergent 3-14. Numbers above indicate p*I* values of IEF p*I* calibration kit.

adult forms represented by two bands with p*I* 6.6 and 6.7 for detergent-solubilized forms and 7.15 and 7.35 for papain-solubilized forms (not shown).

The present findings suggest that desialylation of brush-border membranes plays some physiological role in the redifferentiation process of enterocytes during the postnatal period and that it may be under hormonal control. The hydrocortisone effect on the sialic acid content of brush-border membranes could be different for old and new enterocyte populations. Three days after hormone application both sialylated and desialylated forms of enzymes are present, whereas another 3 days later the desialylated forms prevail. The latter phenomenon could reflect the decreased sialic acid content in the brush borders of newly differentiated cells migrating from the crypts. Also, an interplay between sialylation and desialylation processes in a single cell could explain our findings.

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