Effect of Hydrocortisone on the Development of Embryonic Chick Duodenum —Scanning Electron Microscopic and Enzymatic Studies—

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Summary. The effect of hydrocortisone on morphological changes in developing chick embryo was observed by scanning electron microscopy and light microscopy in relation to changes in enzyme activities.

The morphological changes caused by administration of hydrocortisone were remarkable. In the hydrocortisone-dosed 19th day embryonic duodenum, this hormone caused increases in the height and microvillus length of absorptive epithelial cells, and in the ratio of microvillus length to cell height, up to the normal just hatched 1st day hatched duodenal level. Injections of this hormone also induced precocious increases in maltase and alkaline phosphatase activities.

On the basis of these results, it is considered that hydrocortisone may be one of the important factors regulating normal development in chick embryonic duodenum and may exert a major influence on the growth and maturation of absorptive epithelial cells.

Introduction

It is well known that hydrolytic enzymes of the intestinal microvilli increase their activity at the time of hatching in birds and weaning in mammals^{12, 13, 14, 21, 22}. Morphological observations have also revealed that intestinal mucosa undergoes dramatic changes in size of the villi⁵, height of absorptive epithelial cells⁷, and length of microvilli^{19, 20} at the same time. Recently, Noda (1979) clearly demonstrated that villi and

absorptive epithelial cells of chick duodenum, from the 20th day of incubation to the 2nd day after hatching, developed rapidly with increasing ratio of microvillus length to cell height and changed from the young form to the mature form, and that the activities of alkaline phosphatase and maltase increased remarkably in parallel with microvillus length at this stage¹⁷.

Some reports have indicated that injection of hydrocortisone into developing chick embryo caused a precocious

change in duodenal alkaline phosphatase activity^{1,24)} and a significant increase in disaccharidase activities of the duodenum during organ culture²⁶⁾. These observations suggest that adrenocortical hormone is one of the factors involved in the induction of microvillus hydrolytic enzymes in the duodenum.

The present study was undertaken to elucidate further the role of hydrocortisone in duodenal development. The effect of this hormone on morphological changes in developing chick embryo was examined by scanning electron microscopy and light microscopy in relation to changes in enzyme activities, such as of alkaline phosphatase and maltase.

Materials and Methods

1. *Chick embryos.* White Leghorn fertilized eggs were obtained from the Kazusa Hatchery (Chiba, Japan), and incubated at 37.5°C with 60% relative humidity. On the 14th day of incubation, either 0.25 mg of hydrocortisone sodium succinate dissolved in 0.05 ml of sterile isotonic saline or 0.05 ml of sterile isotonic saline only was injected into the egg yolk via the air sac, and the injection holes in the shell were sealed with scotch tape. The incubation was continued until the 16th, 17th, 18th, 19th or 20th day, at which time the duodena were excised and used for the following experiments.

2. *Assay procedures.* The excised duodena were slit open with a razor blade and used to prepare a 2% homogenate with ice cold redistilled water. Aliquots of the homogenate were subjected to enzyme assay. Alkaline phosphatase activity was determined with p-nitrophenylphosphate as the substrate. The concentration of substrate was 8 mM in 0.5 M 2-amino-2-methyl-1-propanol buffer (pH 10.0)¹⁰⁾. The specific activity

was ultimately expressed as p-nitrophenol released per min per mg of protein using a molar extinction coefficient at 410 nm of 15,300⁴⁾. Maltase activity was determined by the method of Dahlqvist⁹⁾. The concentration of substrate was 28 mM maltose in 0.1 M sodium maleate buffer (pH 6.0). The enzyme activity was expressed as μ moles substrate hydrolyzed per hr per mg of protein. Total protein was determined by the method of Lowry *et al.*¹¹⁾. Bovine serum albumin was used to prepare the standard curve.

3. *Scanning electron microscopy and light microscopy.* The excised duodena were quickly placed in cold 0.1 M sodium phosphate buffer (pH 7.4). A micropipette was used to direct a stream of buffer into the gut to remove mucous. The gut was slit open lengthwise on a filter paper and fixed in 2% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 2 hr. After fixation, the tissues were rinsed in the phosphate buffer and treated by conductive staining as follows. The tissues were immersed in a reagent containing 2% sucrose, 2% sodium glutamate and 2% glycine for 2 hr, in 2% tannic acid for 2 hr, and then after washing in the buffer overnight they were placed in 2% osmium tetroxide for 2 hr²⁶⁾. After the conductive staining, the tissues were rinsed for 1 hr in distilled water, dehydrated in a graded series of ethanol and amylacetate, and then dried in a critical point drier (HCP-1, Hitachi) using CO₂ as the transitional fluid. For examination of absorptive epithelial cells and their microvilli, the upper part of the villi was carefully cut with a razor blade under a dissecting microscope. The length of the microvilli and height of cells of the upper part of the villi showed the largest values for villi observed in a previous study¹⁷⁾. All samples were coated with gold in a

gold vapor coater (Eiko 1B-3). The tissues were examined under a scanning electron microscope (MSM4C-201, Akashi).

Samples for examination by light microscopy were fixed in 10% formalin in 0.1 M sodium phosphate buffer (pH 7.4) at 0°C for 3 hr. After fixation, the tissues were rinsed in running tap water overnight, dehydrated in a graded series of ethanol, and embedded in paraffin. They were then cut at 6 μ , and the sections were stained with either hematoxylin-eosin or the colloidal iron reaction of Mowry¹⁶.

Results

The effect of hydrocortisone on morphological changes was observed, paying particular attention to the size of the villi, the height and microvillus length of absorptive epithelial cells, and the ratio of the microvillus length to cell height, in relation to changes in enzyme activities.

In the light and scanning electron microscopic studies, no effect of hydrocortisone on the size of the villi of the 16th day embryo was observed. In hydrocortisone-dosed 18th and especially 19th day embryos, however, the villi became rapidly larger (Figs. 1-4), the colloidal iron reaction-positive brush border of the villi became more clearly visible (Figs. 3, 4), and goblet cells increased in number and size (Figs. 3, 4), when compared to control embryos which received isotonic saline only.

Two days after administration of hydrocortisone (i.e. in the 16th day embryo), the height of the absorptive epithelial cells increased significantly, when compared to control chick embryos, and this effect became more evident on the 18th day of incubation (Text-fig. 1). In the hydrocortisone-dosed 19th day embryo, a rapid increase in height of

Text-fig. 1. Changes in height and microvillus length of absorptive epithelial cells in hydrocortisone (HC, open circles) - and saline (S, solid circles) - injected chick embryos. The height of the cells and length of the microvilli were measured from scanning electron micrographs such as shown in Figs. 5-8. Each point on the curves represents the mean $\pm$ S.E. of 25-30 cells in three (S) and five (HC) embryos, respectively, with the exception of the 20th day embryo (HC) in which 15-20 cells were measured in two embryos. The 20th day embryos are not discussed in this study because of the high mortality. The difference between HC and S is significant ($p < 0.0001$).

the absorptive epithelial cells was recognized (Text-fig. 1, Figs. 5, 6), and nuclei of the cells were situated near the center of the cytoplasm, as observed at the time of hatching in the normal course of development¹⁷. No effect of hydrocortisone on the length of the

Text-fig. 2. Induction of maltase and alkaline phosphatase in the duodenum of hydrocortisone (HC, open circles)- and saline (S, solid circles)-dosed chick embryos. Each point indicates the mean $\pm$ S.E. of 4-5 duodena. Alkaline phosphatase activity (ordinate) was estimated as μ moles p-nitrophenol produced per min per mg of protein. Maltase activity (ordinate) was estimated as μ moles substrate hydrolyzed per hr per mg of protein. The difference (*) between HC and S is significant ($p < 0.025$). ** $p < 0.01$ *** $p < 0.005$ **** $p < 0.001$

microvilli was observed in the 16th day embryo. However, the effect became apparent in the 18th day embryo, and on the 19th day, the length of the microvilli increased rapidly (Text-fig. 1, Figs. 7, 8), as was also observed at the time of hatching in the normal course of development¹⁷⁾. The ratio of the microvillus length to absorptive epithelial cell height was 1:22-33 in saline-dosed 16th-20th day embryos and

hydrocortisone-dosed 16th-18th day embryos. On the other hand, the ratio was 1:12-14 in hydrocortisone-dosed 19th and 20th day embryos. In the normal course of development, the ratio is 1:20-26 before hatching and 1:15-18 after hatching¹⁷⁾. Thus, in the hydrocortisone-dosed duodenum, the morphological features of the absorptive epithelial cells changed from the young form to the mature form in the 19th day embryo,

i.e. 2 days earlier than in normal development¹⁷⁾.

Hydrocortisone also induced a precocious increase in activities of maltase and alkaline phosphatase as shown in Text-fig. 2. The enzyme activities in the hydrocortisone-dosed embryos showed a rapid increase in the 19th day embryo (5 days after the administration of hydrocortisone), while in the normal course of development, similar changes were observed at the time of hatching¹⁷⁾. The initial increase in maltase activities was already apparent on the 16th day, although the change in alkaline phosphatase activity correlated well with the change in length of the microvilli caused by the hydrocortisone administration. That is to say, the effect was not observed on the 16th day, but it was observed from the 17th day onwards (Text-fig. 2).

Discussion

Administration of hydrocortisone to the 14th day embryo resulted in a precocious induction of maltase and alkaline phosphatase activities and a precocious morphological development of duodenal villi as reflected in increases in size of the villi, in height and microvillus length of absorptive epithelial cells, and in the ratio of microvillus length to cell height. It has been reported that the concentration of hydrocortisone reached its peak in chick embryonic plasma on the 15th day⁹⁾. It appears that the administration of hydrocortisone to the 14th day embryo undertaken in the present study caused a precocious rise in the plasma hydrocortisone level, resulting in precocious induction of enzyme activities and precocious morphological development of the duodenum.

It is worthy of note that the effect of hydrocortisone leading to elongation of

the absorptive epithelial cells and increase in the maltase activities, was observed earlier than that leading to increase in length of the microvilli and enhanced alkaline phosphatase activity. It is well known that maltase and alkaline phosphatase are bound to the microvillus membranes. However, it has been reported that soluble maltase is demonstrated in the cytoplasm of absorptive epithelial cells of suckling rats, and the soluble maltase predominates over the microvillus membrane-bound maltase in the suckling period⁹⁾. The presence of soluble maltase in chick embryonic intestine was not confirmed, but it has been reported that the properties of maltase changed after hatching^{23, 27)}. The maltase induced by hydrocortisone on the 16th day in the present study may be different from microvillus membrane-bound maltase: it may be soluble maltase in the cytoplasm of absorptive epithelial cells since the microvillus length did not increase at this stage. As shown in Text-fig. 2, the second upsurge in maltase activity induced by hydrocortisone correlated well with the increase in length of the microvilli.

On the basis of these results, it is considered that hydrocortisone may be one of the important factors regulating normal development in chick embryonic duodenum and may exert a major influence on the growth and maturation of absorptive epithelial cells.

Repletion of vitamin D causes a subsequent increase in length of the microvilli of duodenal cells of chicks⁹⁾ and rats¹⁸⁾. Embryonic chick tissue possesses enzymes necessary for the synthesis of the active form of vitamin D₃(1,25-(OH)₂D₃), but accumulation of this active form is not possible in the intestine until just before hatching¹⁵⁾. Carré *et al.* (1974) have reported that adrenal corticoids can alter the metabolism of 1,25-(OH)₂

D₃²⁹. From the present study, it appears that 1,25-(OH)₂D₃ may exert an influence on the precocious elongation of microvilli, but the details of the mechanism by which hydrocortisone acts on 1,25-(OH)₂D₃ are unknown. This possibility will be investigated further in future studies.

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Explanation of Figures

Plate I

Figs. 1 and 2. Scanning electron micrographs of duodenal villi of 19th day chick embryos which had received an injection of sterile isotonic saline (Fig. 1) or hydrocortisone (Fig. 2). The villi in Fig. 2 show a remarkable increase in size over those in Fig. 1. ($\times 190$)

Figs. 3 and 4. Light micrographs of duodenal villi of 19th day chick embryos which had received an injection of sterile isotonic saline (Fig. 3) or hydrocortisone (Fig. 4), stained with Mowry's colloidal iron reaction for acid mucopolysaccharides. The colloidal iron reaction along the surface of epithelial cells and in goblet cells is far more prominent in Fig. 4 than in Fig. 3. ($\times 260$)

Plate II

Figs. 5 and 6. Scanning electron micrographs of duodenal absorptive epithelial cells of 19th day embryos which had received an injection of saline (Fig. 5) or hydrocortisone (Fig. 6). The effect of hydrocortisone on the height of cells is remarkable, compared to the control. ($\times 2,600$)

Plate III

Figs. 7 and 8. Scanning electron micrographs of microvilli of duodenal absorptive epithelial cells of 19th day embryos which had received an injection of saline (Fig. 7) or hydrocortisone (Fig. 8). The length of the microvilli is markedly increased in the hydrocortisone-dosed embryo. ($\times 31,000$)