PREMATURE INDUCTION OF PEPSINOGEN IN DEVELOPING RAT GASTRIC MUCOSA BY HORMONES

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SUMMARY

Daily injection of ACTH into rats from the age of 9 days caused precocious development of peptic activity in the gastric mucosa. A single injection of hydrocortisone into rats aged 2 to 9 days also evoked premature development of peptic activity in the gastric mucosa. The patterns on polyacrylamide gel electrophoresis of pepsinogen from adults and from infants treated with hormone were quite similar but differed from that of untreated infants. Enzyme from the gastric mucosa of adult rats and enzyme evoked with hormones were more stable than that from infant rats.

INTRODUCTION

Little is known about the process of differentiation in the gastric glandular mucosa. In adult animals pepsinogen apparently comes from the chief cells and the mucous neck cells (1, 2). The frequent presence of morphologically intermediate forms between the chief cells and mucous neck cells in rats during the first 10 days after birth has been reported (3).

The present paper describes changes in the activity of pepsin in the mucosa of the glandular stomach of rats and changes in the molecular species of pepsinogen up to the 30th day after birth demonstrated by polyacrylamide gel electrophoresis. The effects

of the hormones, ACTH and hydrocortisone in evoking premature development of pepsin activity are also described.

MATERIALS AND METHODS

Rats were of the Wistar-Imamichi inbred strain. The stomach was quickly removed from animals anesthesized with ether. The gastric mucosa was scraped from the muscle layer with a microscope cover glass and was homogenized with 9 volumes of ice cold 0.02 M KH_2PO_4 and $NaHCO_3$ (pH 7.0) in a Potter-Elvehjem glass homogenizer with a teflon pestle at 1000 r. p. m. for 2 min. The body weights of adult rats were 200 ± 10 g. All infant rats were nursed by their mothers and began taking solid food after 18 days.

The potential peptic activity of pepsinogen in the mucosal homogenate was determined by the method of Anson (4) with a slight modification. Enzymatic activity was measured in dilute HCl solution (pH 1.8) containing 1.67 % bovine hemoglobin (Sigma, type I). The reaction was allowed to proceed for 5 or 10 min at 37°C after addition of the mucosal homogenate. The digestion of denatured hemoglobin was estimated by measuring the absorbance at 280 nm of the trichloroacetic acid soluble fraction. Protein was measured by the method of Lowry *et al.* (5).

Synthetic ACTH (Organon and Co.) was injected intramuscularly. Hydrocortisone acetate (Merck and Co.) was injected subcutaneously into the back. Control animals received injections of saline.

Electrophoresis of pepsinogen was performed by the method of Samloff *et al.* (6) with the following modifications. Polyacrylamide gel (7.5 and 5.0 %) and 0.05 M tris-acetate buffer (pH 8.2) (Ref. 7), were used instead of agar gel and 0.05 M veronal buffer, respectively. The supernatant after centrifugation of the homogenate at 100,000 x g was applied as the enzyme extract. Recovery of peptic activity after this centrifugation was more than 90 %.

RESULTS

Fig. 1a illustrates the change in peptic activity in the gastric mucosa of rats from birth. Up to the 17th day after birth, the peptic activity of the pepsinogen in the gastric mucosa remained constant at approximately 20 % of that of adult rats. Peptic activity increased from the 18th day, reaching the adult level on the 30th day.

As shown in Fig. 1b when ACTH was injected three times intramuscularly at the rate of 80 units/Kg/day from the 9th day after birth, the peptic activity began to increase on the 11th day and reached the adult level on the 13th day. Then the peptic activity decreased sharply on the 14th day and remained at about half the adult level for

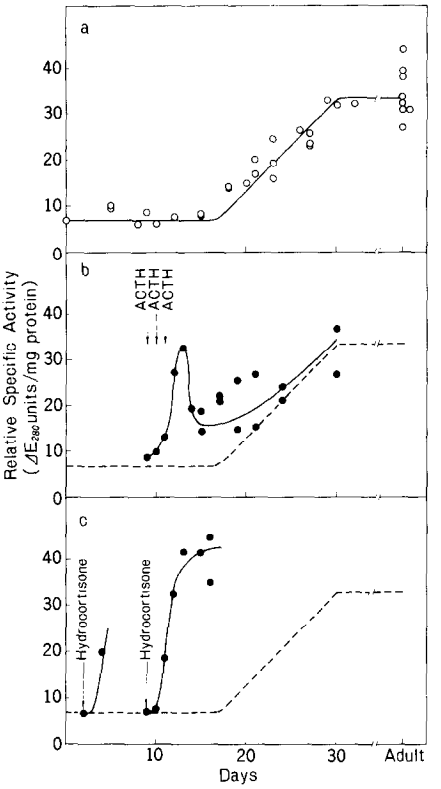
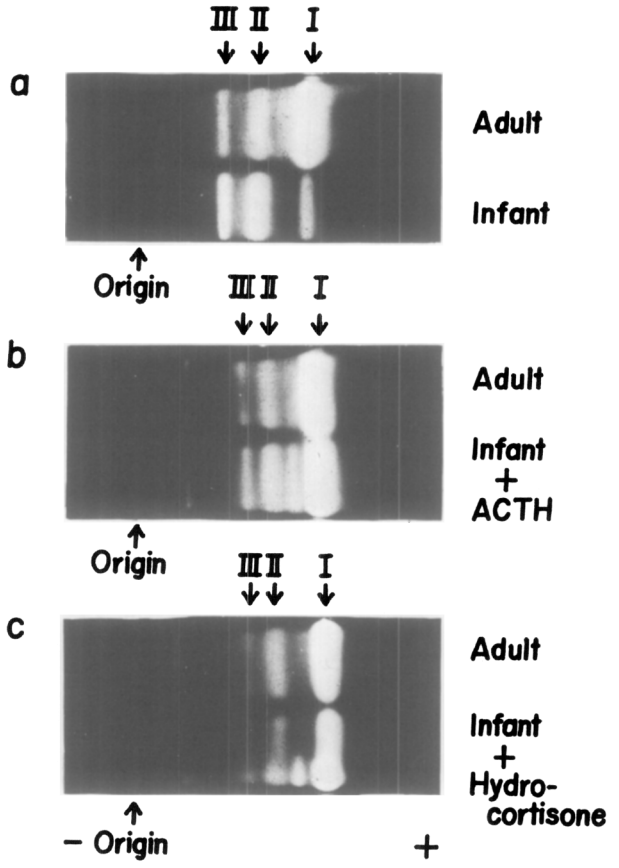


Fig. 1 a) Change in the level of pepsinogen in the gastric mucosa during development. Each point is the mean of triplicate assays. Each point before the 15th day is the mean of triplicate assays of a pool of 5 to 10 stomachs of infants of the same litter. b) Premature formation of pepsinogen in the gastric mucosa evoked by ACTH. ACTH was injected intramuscularly once daily (80 units/Kg) from the 9th to the 11th day. The dashed line indicates the normal developmental process. The arrow shows the time of injection of ACTH. c) Premature formation of pepsinogen in the gastric mucosa evoked by hydrocortisone. Hydrocortisone was injected subcutaneously into the back (250 mg/Kg) on the 9th day. Dashed line, control.

several days. Then it increased again, closely following the curve observed during normal development. When the daily injections of ACTH at the rate of 80 units/Kg/day from the 9th day were continued, the peptic activity remained at a high level at least until the 21th day.

As illustrated in Fig. 1c when hydrocortisone acetate (250 mg/Kg) was injected once on the 9th day after birth, a similar precocious increase in the peptic activity occurred after a latent period of 2 days. This hormone was capable of eliciting the



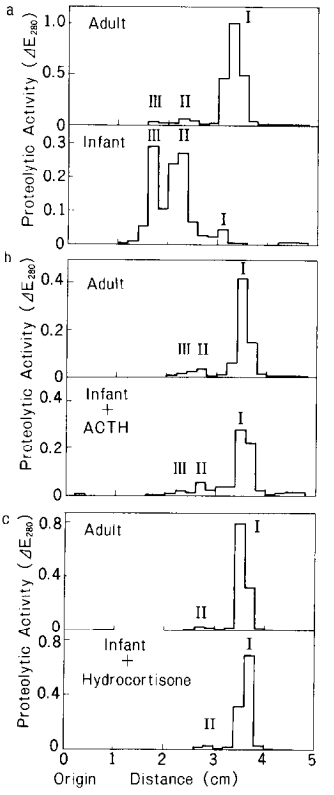


Fig. 2 Electrophoresis of pepsinogens on polyacrylamide gel. a) Pepsinogens of adult and infant (10 days old) rats were run in 7.5 % polyacrylamide gel under 24 mA/2.7 cm^2 and 90V/11.1 cm for 2.5 hr. After electrophoresis, one part of the gel was immersed in a solution of 0.65 % hemoglobin in 0.06 N HCl for 10 min, incubated in a humid chamber at 37°C for 1 hr and stained with 0.1 % amide black 10B in 7 % acetic acid for 5 min (Fig. 2 Aa). The other part of the gel was frozen and cut into 2 mm widths. Each strip was chopped into small pieces and put in 0.1 ml of 0.075 N HCl at 4°C. Proteolytic activity of each sample was measured with hemoglobin as described in MATERIALS AND METHODS. The reaction was allowed to proceed for 36 min with infant pepsinogens and for 12 min with adult pepsinogens (Fig. 2 Ba). b) Pepsinogens of the adult control and of the infants (13 days old) treated with ACTH in 5 % polyacrylamide gel. Conditions were the same as in a) but electrophoresis was for 2.0 hr. The reaction was carried out for 12 min with both pepsinogens (Fig. 2 Bb). c) Pepsinogens of the adult control and of the infants (14 days old) treated with hydrocortisone in 5 % polyacrylamide gel. Conditions were the same as in b). The reaction was carried out for 12 min with both pepsinogens (Fig. 2 Bc).

peptic activity even in animals as young as 2 days old. However, it was not effective in one day old animals.

Male and female animals showed a similar normal developmental process and response to hormones. Similar treatment of adult rats with the hormones had no effect.

Fig. 2 represents the electrophoretic heterogenity of pepsinogens in the rat gastric mucosa. At least six bands were detected with pepsinogens from adults and infants treated with hormones. The fastest moving band (I) was the main pepsinogen in these animals. Contrary to this, only four bands of pepsinogens were found on normal infant rats. The main components in infant rats were completely different from those in adults (Fig. 2a). The amounts of the main pepsinogens, II and III of infant rats decreased during development or treatment with hormones, while the amount of the minor pepsinogen, I of infant rats increased about 50 to 100 times.

Fig. 3 shows the stability of pepsin in crude extracts of gastric mucosa of rats in 0.02 N HCl at 37°C. Fig. 3a indicates that pepsin from adult rats is more stable than pepsin from infant rats. A mixture of equal volumes of extracts from adults and infants, respectively showed the same peptic activity as the sum of the activities in twice-diluted extracts from the infants and adults. The stability curve of this mixture of extracts indicated that the enzyme from the adult and that from the infant are different, supporting

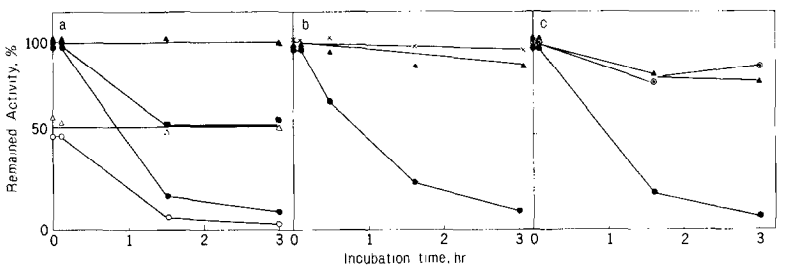


Fig. 3 Stability of pepsin in 0.02 N HCl (pH 1.8) at 37°C. a) The homogenate of the adult rat mucosa was diluted and activated with 160 volumes of 0.02 N HCl (pH 1.8) and assayed as described in MATERIALS AND METHODS after incubation for appropriate times at 37°C. The homogenate of the infant rat (15 day old) mucosa was diluted and activated with 25 volumes of 0.02 N HCl (pH 1.8). b) The mucosal homogenate of ACTH treated infants (14 day old in Fig. 1b) was diluted and activated with 140 volumes of 0.02 N HCl. The homogenate from the adult was treated with 130 volumes of 0.02 N HCl and that from the infants (14 day old) was diluted with 20 volumes of 0.02 N HCl. c) The mucosal homogenate of hydrocortisone treated infants (16 day old in Fig. 1c) was diluted and activated with 190 volumes of 0.02 N HCl. The homogenate from the adult was diluted with 100 volumes of 0.02 N HCl and that from the infants (12 day old) with 25 volumes of 0.02 N HCl. ▲, adult; ●, infant; △, adult diluted twice; ○, infant diluted twice; ■, mixture of 1 volume of adult and 1 volume of infant extract; ×, infant + ACTH; ◎, infant + hydrocortisone

the results obtained by electrophoresis. Fig. 3b and 3c show that the enzyme activity evoked by hormones has similar stability to the enzyme from the adult.

DISCUSSIONS

The change in peptic activity in a homogenate of the whole stomach of developing rats was previously studied by Boass et al. (8) and Helander (3). We confirmed that peptic activity increased during the late suckling period using a homogenate of mucosa from the glandular stomach. ACTH or hydrocortisone injection in the earlier stage of development induces formation of some enzymes which normally develop during the late suckling period. These are invertase in the intestine (9), and glucokinase (10), tryptophan oxygenase (11) and ornithine aminotransferase (12) in the liver.

The present study showed that ACTH and hydrocortisone evoked premature pepsinogen formation. Figs. 2 and 3 show that these hormones evoked formation of the enzyme found in the adult. This suggests that hydrocortisone is related to cell maturation with respect to formation of particular molecular species of enzyme. Formation of pepsinogen was not stimulated by injection of hydrocortisone into new born or one day old animals and the animals became sensitive to hydrocortisone stimulation on the 2nd day after birth. The second state after birth is a period from the age of 2 to 17 days. The pepsin which is found normally during this period, is more labile than that in adults. Injection of hydrocortisone or ACTH during this period was very effective. The third stage after birth is a period from the age of 18 to 30 days, when the normal maturation process takes place.

The difference in the molecular species of pepsinogen found in infants and adults suggests that in infants the main acid proteases are produced in the mucous neck cells while in adults the main acid protease is synthesized in the chief cells, because it is reported that the appearance of chief cells (3) coincides with elevation of pepsin activity and with a change in its molecular species.

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