Acta Medica Okayama

Volume 48, *Issue* 1 1994 *Article* 8

FEBRUARY 1994

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Abstract

The effects of epidermal growth factor (EGF) on neonatal intestines were examined in the rat. In 5-day-old rats, sucrase, trehalase, alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (gamma-GTP) activities in the small intestines were significantly increased after subcutaneous injection of EGF for 3 days (1 microgram/rat/day). gamma-GTP activity was also accelerated after oral EGF administration (2 micrograms/rat/day). Small intestines of 12-day-old rats injected with EGF for 10 days (1 microgram/rat/day) were significantly heavier than those of controls. These results suggest that EGF influences neonatal growth improving enlargement and functional development of their intestines.

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*PMID: 7910714 [PubMed - indexed for MEDLINE]

ACTA MED OKAYAMA 1994; 48(1): 47-50

Effects of Epidermal Growth Factor on Neonatal Growth of Rat Intestines

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The effects of epidermal growth factor (EGF) on neonatal intestines were examined in the rat. In 5-day-old rats, sucrase, trehalase, alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (γ-GTP) activities in the small intestines were significantly increased after subcutaneous injection of EGF for 3 days (1 μg/rat/day). γ-GTP activity was also accelerated after oral EGF administration (2 μg/rat/day). Small intestines of 12-day-old rats injected with EGF for 10 days (1 μg/rat/day) were significantly heavier than those of controls. These results suggest that EGF influences neonatal growth improving enlargement and functional deveopment of their intestines.

Key words: epidermal growth factor, neonate, intestine, breast milk, rat

Epidermal growth factor (EGF) is a 53-amino acid polypeptide that was first isolated from the submandibular glands of adult male mice in 1962 (1), and it has a mitogenic effect on a wide variety of cells of ectodermal and mesodermal origin (2). Numerous studies have revealed its morphological effects on various tissues *in vivo* and *in vitro*. However, there have been few investigations of its influence on the physiological functions of those tissues. One of the most specific biological actions of EGF is precocious eyelid opening in newborn mice following its administration (1). This effect is routinely observed after subcutaneous injection, but it has also been produced by oral EGF administration (3).

EGF is present in many mammalian tissues and body fluids, including blood, saliva, and breast milk (2, 4). Both human and rodent breast milk contains EGF in high level; moreover, it is more abundant in colostral than in mature milk (5, 6). Carpenter (7) found that the mitogenic activity of milk was neutralized *in vitro* by the addition of human EGF antibody and characterized EGF as a major growth-promoting agent in milk. Orally administered ^{125}I-EGF was shown to have degraded to only a small extent in the stomach and small intestinal lumen of the suckling rat (8). It has been suggested that the luminal EGF content of the suckling rat is highly dependent on milk intake (9).

We previously reported that EGF played some important roles in human and rodent fetal growth (10-12). We also suppose that breast milk, which contains much EGF, plays an important role in neonatal growth. In the present study, we examined the effect of EGF on the neonatal growth, especially on the rat intestine.

Materials and Methods

Experimental procedures. Newborn Wistar rats were obtained from a breeding colony at Okayama University Medical School and used in the EGF administration studies. They were housed at 22 $\pm$ 2°C in a light-controlled room with a 12 h/12 h light-dark cycle with their mothers. One half of the pups were treated with mouse EGF (Wako Pure Chemicals Industries, Osaka, Japan), and the other half comprised the control group.

In the subcutaneous administration studies, pups in the experimental group were injected daily with 1 μg of EGF in 0.05 ml of saline for 3 or 10 days from day 2 of life. Littermate controls were injected with an equivalent volume of saline. In the oral administration study, starting on day 2 of life, pups were given either 2 μg of EGF in 0.1 ml of H_2O or H_2O alone via a 24-gauge soft catheter for 3 days.

On day 5 of life, pups injected with EGF or saline for 3 days were weighed and sacrificed by decapitation. The small intestines were immediately removed, rinsed with cold saline, and homogenized in 10 vol of ice-cold saline

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in a glass-Teflon homogenizer. The homogenates were centrifuged at 2,000 × *g* for 15 min, and the supernatants were removed for enzymatic determinations. Protein concntrations and the activities of sucrase, trehalase, lactase, maltase, alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (γ-GTP) were measured. The homogenates were also used for DNA determinations. Orally treated pups were also sacrificed on day 5, and their small intestines were removed for ALP, γ-GTP and protein measurement.

On day 12 of life, pups injected with EGF or saline for 10 days were weighed and decapitated and the visceral organs were removed. The stomach and intestine were opened, rinsed, blotted dry, and weighed. The liver was weighed after blotting to remove surface blood.

Enzyme activities. The activities of disaccharidases, (*i.e.*, sucrase, trehalase, lactase, and maltase) were assayed according to the method of Dahlqvist (13). In the reported results, units of activity (U) = μmol disaccharide hydrolyzed/min at 37°C.

ALP activity was measured with an Alkaline Phospha B-Test (Wako Pure Chemicals Industries). γ-GTP activity was determined with a γ-GTP C-Test (Wako Pure Chemicals Industries).

DNA and protein concentrations. The DNA concentration was determined according to the method of Schneider (14), with calf thymus DNA (Sigma Chemical Co., St. Louis, MO, USA) used as the standard. The protein concentration was determined by the method of Lowry *et al.* (15).

The results are reported as means ± SD. Statistically significant differences between mean values were determined by Student's *t*-test.

Results

The body weight of 5-day-old rats subcutaneously given EGF for the preceding 3 days did not differ significantly from that of controls (10.2 ± 2.0 g *vs.* 10.5 ± 1.9 g). The effect of EGF on the small intestines of these animals is shown in Table 1. The protein content was significantly elevated ($p < 0.05$) in the EGF-treated group, but there was no significant change in DNA content. Sucrase and trehalase activities were increased significantly in the EGF-treated group compared with the control group ($p < 0.02$ and $p < 0.01$, respectively). However, lactase and maltase activities were not altered significantly by EGF administration. ALP activity was significantly higher ($p < 0.05$) in the EGF-treated group than in the control group, as was γ-GTP activity ($p < 0.001$).

Table 1 Protein and DNA content and enzyme activities in small intestines of control and EGF-treated 5-day-old rats

	Control (n)	EGF-treated (n)
Protein content	97.1 ± 10.1(19)	104.7 ± 12.3(19)[a]
DNA content	3.99 ± 0.81(14)	4.11 ± 0.67(13)
Enzyme activities		
Sucrase	0.37 ± 0.10(14)	0.51 ± 0.17(14)[b]
Trehalase	2.27 ± 0.71(18)	3.23 ± 1.10(19)[c]
Lactase	88.5 ± 12.1(18)	84.3 ± 17.0(18)
Maltase	51.0 ± 10.6(18)	54.0 ± 12.8(19)
ALP	704.2 ± 130.5(19)	817.2 ± 198.6(19)[a]
γ-GTP	37.6 ± 10.3(19)	54.7 ± 11.2(19)[d]

Values are means ± SD of mg/g wet tissue (protein, DNA), U/g protein (sucrase, trehalase, lactase, maltase) or IU/g protein (ALP, γ-GTP). [a] $p < 0.05$ *vs.* control. [b] $p < 0.02$ *vs.* control. [c] $p < 0.01$ *vs.* control. [d] $p < 0.001$ *vs.* control.
EGF: epidermal growth factor; ALP: alkaline phosphatase; γ-GTP: γ-glutamyl transpeptidase.

Table 2 ALP and γ-GTP activities in small intestines of 5-day-old control rats and rats given EGF orally

Enzyme	Control (n)	EGF orally administered (n)
ALP	688.9 ± 129.3(13)	719.6 ± 123.4(14)
γ-GTP	40.6 ± 13.9(13)	55.3 ± 15.5(14)[a]

Values are means ± SD of IU/g protein.
[a] $p < 0.02$ *vs.* control.

We added oral EGF administration for the study of ALP and γ-GTP activities because the possibility existed that subcutaneously administered EGF might affect intestinal enzymes via stimulation of some other metabolic systems. As shown in Table 2, γ-GTP activity was significantly increased ($p < 0.02$) following oral EGF administration as well. ALP activity in the EGF group was slightly, but not significantly, enhanced.

Daily subcutaneous injection of EGF for 10 days, starting at 2 days of age, did not modify body weight (Table 3). However, the small intestinal weight of the EGF-treated group was significantly heavier ($p < 0.001$) than that of the control group (Table 3). The stomach and liver weights of the EGF-treated group showed a similar tendency.

Table 3 Body and organ weights of control and EGF-treated 12-day-old rats

	Control (n = 13)	EGF-treated (n = 13)
Body weight (g)		
Day 2	6.9 ± 0.3	6.9 ± 0.4
Day 12	20.0 ± 3.3	20.3 ± 3.1
Gain	13.1 ± 3.2	13.4 ± 3.0
Organ weight (mg)		
Small intestine	481 ± 59	605 ± 80[a]
Stomach	148 ± 28	159 ± 26
Liver	494 ± 100	545 ± 103

Values are means ± SD. EGF: See Table 1.
[a] $p < 0.001$ *vs.* control.

Discussion

In this study, the activities of the intestinal brushborder enzymes such as sucrase, trehalase, ALP, and γ-GTP were significantly increased in 5-day-old suckling rats after the subcutaneous injection of EGF for the preceding 3 days. However, lactase and maltase activities were not affected by EGF administration. In addition, we observed orally administered EGF enhanced γ-GTP activity in rats of the same age.

Previous studies have shown that receptors for EGF are present in the gastrointestinal tract and have been localized to both brush-border (16) and basolateral (17) membranes. In the adult rat, intravenously injected ^{125}I-labeled rat EGF was cleared from the circulation within minutes, and most of the label was distributed in the liver (52 %), kidneys (14 %), small intestine (11 %), and skin (7 %). In the small intestine, the label was found in the surface epithelium of the villi by autoradiography (18). Moreover, in 13- to 15-day-old suckling rats, orally administered ^{125}I-EGF was absorbed largely (65 % of fed label), distributed to the intestinal wall (5 % of fed label), and remained in intact form (3.5 % of fed label) in the intestinal wall (19). Thus, we speculate that both subcutaneous and oral administration of EGF influences the intestines.

There have been few reports concerning the influence of EGF on intestinal brush-border hydrolytic activities in suckling animals. Oka *et al.* reported that EGF, administered subcutaneously in a dose of 0.1 μg/g body weight twice daily for 3 days, increased lactase activity in 2-week-old suckling rats, but had no effect on maltase or sucrase activity (20). The difference in the effects of EGF on disaccharidase activities may be due to developmental differences between 2-day-old and 2-week-old rats. Malo *et al.* treated 8-day-old suckling mice with subcutaneous injection of EGF at a dose of 1 or 4 μg/g body weight/day for 3 days and observed the premature appearance of sucrase activity and increased trehalase, glucoamylase, lactase, ALP, and γ-GTP activity in the intestine (21). These results suggest that EGF may play a role in neonatal growth by regulating intestinal absorption through enhancement of brush-border enzyme activities.

It was reported that breast milk-fed animals had significantly heavier intestines, livers and kidneys than animals fed artificial formula (22). The concentration of EGF in breast milk is 100- to 1,000-fold higher than plasma; for example, it is 200-400 ng/ml in mouse milk, 1 ng/ml in mouse plasma, 30-340 ng/ml in human milk, and 0.1-0.2 ng/ml in human plasma (4, 5, 23). Rat milk contains approximately 40 ng/ml of EGF (8). Hence, our data suggest that EGF, abundant in breast milk, influences functional maturation of neonatal intestines from as early as 2-4 days of life.

We also noted a significant increase in intestinal weight after daily subcutaneous injection of EGF for 10 days, which agrees with the data reported by Oka *et al.* (20). EGF is wellknown for its proliferative effect on various kind of cells. Ornithine decarboxylase (ODC), the rate-limiting enzyme of the polyamine biosynthesis pathway, is well-known to show increased activity in conjunction with cell proliferation. In the study of primary cultured rat hepatocytes, EGF markedly increase the activity of ODC (24). EGF, administered subcutaneously, has been shown to stimulate ODC activity in the liver of suckling rats (12), and the stomach and intestine of suckling mice (25). Berseth reported that newborn rats fed artificial formula which contained 1.2, 3.0, and 6.0 μg/ml EGF every 3 h for 39 h had greater DNA synthesis and DNA content in the intestine than controls fed unsupplemented formula (26). Thus, EGF probably has a proliferative effect on the digestive tract.

It is concluded that EGF, abundant in breast milk, stimulates the growth and functional development of neonatal rat intestines.

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Received June 23, 1993; accepted Septemper 6,1993.