PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 159, 400–402 (1978)

Epidermal Growth Factor Stimulates Ornithine Decarboxylase Activity in the Digestive Tract of Mouse (40357)

EDWARD J. FELDMAN, DOROTHEA AURES, AND MORTON I. GROSSMAN

VA Wadsworth Hospital Center and UCLA School of Medicine, Los Angeles, California

Urogastrone (UG), extracted from human urine, and epidermal growth factor (EGF), extracted from mouse salivary glands, are polypeptides that have the same biologic actions and are highly homologous in amino acid sequence (1). Both molecules have 53 amino acid residues of which 37 are identical. It is reasonable to assume that the differences in amino acid sequence between UG and EGF are species differences and that within any one species urinary UG and salivary EGF will probably be found to be identical.

In 1938, Sandweiss and colleagues, noting that pregnant women have a low incidence of duodenal ulcer disease, demonstrated that extracts from the urine of pregnant women promoted the healing of experimentally produced (Mann-Williamson) ulcers in dog (2). Soon afterwards, urine extracts from normal men and women as well as from pregnant women were shown to contain a potent inhibitor of gastric acid secretion to which the name urogastrone was given (uro-urine, gastr-stomach, one-inhibitor) (3).

In 1975, H. Gregory reported the amino acid sequence of purified urogastrone (1). He recognized that urogastrone was highly homologous with another polypeptide, epidermal growth factor, described by Savage and Cohen in 1972 (4).

Epidermal growth factor stimulates proliferation and keratinization of epidermal tissue and promotes precocious eye opening and tooth eruption in neonatal mice. In addition, EGF has been shown to stimulate epithelial cell proliferation in cultured chick, mouse and human cells (5). Finally, EGF has been shown to increase L-ornithine carboxy-lase (EC 4.1.1.17) activity in mouse skin (6). This enzyme, ornithine decarboxylase, is an important step in the biosynthetic pathway of the polyamines–putrescine, spermidine and spermine (7). Polyamine production is an index of tissue growth since induction of these substances is closely related to the burst of intracellular activity preceding actual cell synthesis.

Mouse salivary gland EGF and human urinary UG share all of the biologic actions for which they have been tested. Thus, mEGF inhibits gastric acid secretion as effectively as hUG in rats and dogs. Conversely, hUG is equipotent with mEGF in causing precocious eye opening in newborn mice and in stimulating uptake of an amino acid and in displacing labeled UG or EGF from receptor sites in cultured human fibroblasts (8).

Since UG has certain gastrointestinal actions such as inhibition of gastric acid secretion and stimulation of healing of experimental ulcers, it seems reasonable to inquire whether UG and EGF stimulate epithelial growth of the gastrointestinal tract as they do in the epidermal structures.

To examine this question, Stastny and Cohen's model of induction of ornithine decarboxylase by mouse EGF in neonatal mice was employed (6).

Materials and methods. Eight day old mice paired by weight from the same litter were injected subcutaneously on the dorsal surface using a 27 gauge needle with either mEGF (6 μg g^{-1} body wt in water given as a solution containing 220 μg ml^{-1}) or an equivalent volume of water for the control animals. The EGF used was generously provided by H. Gregory, ICI Pharmaceuticals, England. The mice were then returned to their mother where apparent normal feeding patterns continued.

Four hours later the animals were killed by cervical compression and 10–20 mg tissue samples were removed for study from the stomach (whole organ), duodenum (pylorus to 2 cm distal), midgut (from 7 to 10 cm distal to pylorus), colon (mid-cecum to rectum) and heart. The samples were homogenized in all glass tissue grinders (Ten Boeck type) in 50 m*M* sodium–potassium phosphate buffer (9 vol g^{-1}), pH 7.2, containing 1 m*M* EDTA

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(ethylenediamine-tetraacetic acid–disodium salt) and 5 m*M* dithiothreitol, then centrifuged at 100,000*g* for 15 min. Samples from the supernatant were added to incubation tubes containing 0.2 m*M* pyridoxal-5-phosphate, 0.5 m*M* L-ornithine and 0.5 μCi DL 1-[^{14}C]ornithine in a total volume of 0.5 ml of the same buffer. "Blanks" were without tissue extract or with heat inactivated tissue extract. To trap released CO_2, a plastic cup containing a small piece of cotton impregnated with 0.2 ml of 0.5 *M* Protosol (New England Nuclear), a strongly alkaline tissue solubilizer was supported above the incubate by a glass nail. The system had an air tight seal and was incubated at 37°. To insure complete CO_2 release, the incubation mixture was acidified by adding 0.5 ml of 0.5 *N* $HClO_4$ for 60 min. The cups were then transferred to liquid scintillation vials and counted. CPM's were converted to equivalent quantities of CO_2 and expressed as pmoles of CO_2 liberated from 1-[^{14}C]L-ornithine per mg protein or tissue wet weight per hour incubation. Student's paired *t* test was used for statistical analysis.

Results. Validation. Figure 1 demonstrates a linear relationship between quantity of various tissues studied and enzyme activity and Fig. 2 shows a linear relationship between the duration of incubation and enzyme activity. The colon demonstrated a non-linear activity increase after 20 min incubation time.

Alpha-methyl-ornithine, a competitive inhibitor of L-ornithine decarboxylase was used to establish the specificity of the enzyme from the various tissues (9). To produce 50% inhibition under our incubation conditions, the α-methyl-ornithine concentrations required were: stomach, 4×10^{-3} *M*; duodenum and midgut, 1.8×10^{-3} *M*; heart, 1.5×10^{-3} *M*; and colon, 4×10^{-3} *M*.

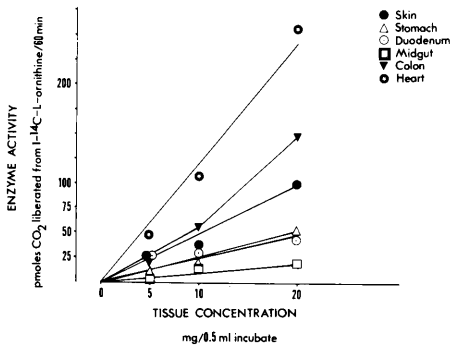

FIG. 1. Relation between enzyme activity (pmoles CO_2 liberated from 1-[^{14}C]L-ornithine per 60-min incubation) and concentrations of tissue homogenates from stomach, duodenum, midgut, colon, heart and skin.

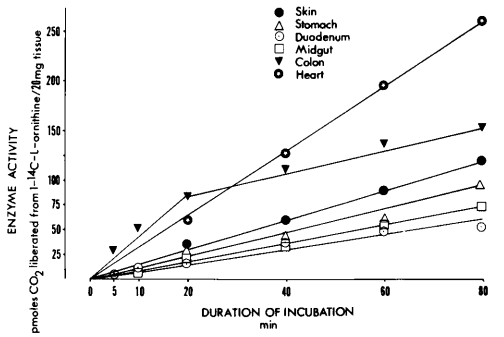

FIG. 2. Relation between enzyme activity (pmoles CO_2 liberated from 1-[^{14}C]L-ornithine per 20 mg tissue samples) and duration of incubation for the same tissues as in Fig. 1.

Initial experiments using homogenates of ventral surface skin demonstrated a significant rise in ornithine decarboxylase (13.0 ± .61 nmoles CO_2 liberated from 1-[^{14}C]L-ornithine per mg protein in the EGF group versus 9.2 ± .56 in the control group; $N = 10$, $P < .01$), confirming the results of Stastny and Cohen.

mEGF experiment. Results are shown in Fig. 3. In the animals pretreated with mEGF there was a significant elevation of ornithine decarboxylase activity in two tissues, the stomach and the duodenum. The increases in the midgut and the colon were not statistically significant. The control tissue, heart, demonstrated no difference.

Discussion. From these results it is concluded that EGF, and therefore probably also UG, stimulates an increase in ornithine decarboxylase activity in the stomach and duodenum of neonatal mice. This suggests a possible physiologic role for EGF in controlling mucosal growth in the proximal digestive tract.

It is of interest that in the control tissue, heart, ornithine decarboxylase can be induced by another stimulus, stress, in the form of aortic constriction (10).

A further hypothesis is suggested from this study. Human urogastrone has been identified by immunofluorescent techniques in the salivary glands and duodenal Brunner's

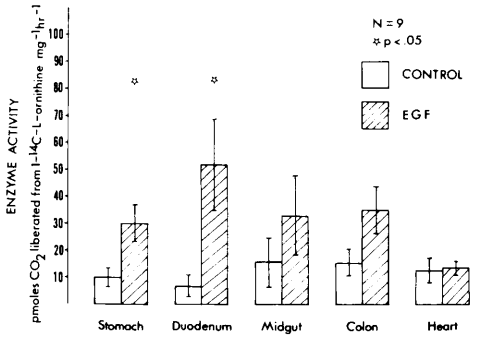

FIG. 3. Enzyme activity (pmoles CO_2 liberated from 1-[^{14}C]L-ornithine per mg wet weight tissue per hour incubation) 4 hr after subcutaneous administration of mEGF (6 μg g^{-1} body wt) or equivalent volume of water.

glands of man (11). This latter location is the most common site for peptic ulceration. Since an increase in secretion of acid and pepsin is not present in many ulcer patients, a decrease in a hypothetical "tissue resistance factor" is assumed to be involved. The nature of this factor is not clear but this study suggests that urogastrone should be considered as a candidate for this role.

Summary. This study examined the effect of EGF (6 μg g^{-1} body wt, subcutaneously) on OD concentration in stomach, duodenum, midgut and colon, as well as a control tissue, heart, in 8-day-old mice. The animals were killed 4 hr after either EGF or control water injections. OD activity, expressed as picomoles of $^{14}CO_2$ liberated from 1-[^{14}C]L-ornithine per mg wet weight tissue, was significantly higher in the animals given EGF than in controls in the stomach (EGF 29.9 ± 6.8; control 9.9 ± 3.6, $P < .05$) and the duodenum (EGF 51.7 ± 16.9; control 6.5 ± 4.3, $P < .05$) but not in the midgut, colon or heart. It is concluded that epidermal growth factor stimulated ornithine decarboxylase activity in the stomach and duodenum of neonatal mice suggesting a possible role for EGF (or urogastrone) in mucosal repair and defense in these tissues.

This study was supported by the National Institute of Arthritis, Metabolism and Digestive Disease Grant 17328 to CURE (Center for Ulcer Research and Education), and by a Veterans Administration Senior Medical Investigatorship (M. I. Grossman) and Research Associateship (E. J. Feldman). We thank Ruth Abercrombie and Kuwa Chou for assistance in preparation of the manuscript and illustrations.

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Received July 24, 1978. P.S.E.B.M. 1978, Vol. 159.