JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY, 1992, 43, 1, 31—39.

ORNITHINE DECARBOXYLASE IN LARGE BOWEL MUCOSA: REGULATION BY GASTRIN, SECRETIN AND EGF

L. R. JOHNSON, P. WANG, K. HADDOX*

Department of Physiology and Biophysics University of Tennessee College of Medicine Memphis, TN
*Deparment of Pharmacology University of Texas Medical School Houston, Texas

Rats were fasted 48 h and then injected once with either saline, pentagastrin, EGF, secretin or combinations of secretin and pentagastrin or EGF. Another group of rats was fasted and refed. Animals were killed 4 h later and ODC assayed in mucosa of the cecum, proximal colon, and distal colon. EGF significantly increased ODC activity in all 3 tissues. Secretin had no effect by itself on ODC or ODC stimulated by EGF. Pentagastrin significantly increased ODC of the cecum, and secretin completely inhibited the effect of pentagastrin. Refeeding fasted rats significantly induced activity in all three tissues. Immunocytochemistry using a highly specific polyclonal ODC antibody showed that ODC was confined to the crypt cells of the proximal colon. Antibody dilution techniques demonstrated that gastrin, EGF and refeeding increased the level of enzyme in these cells. Refeeding in addition caused the appearance of enzyme in surface epithelial cells. These results showed that colonic mucosal ODC is present in proliferative cells and is regulated by the same peptides known to regulate growth in this tissue. Colonic mucosal ODC also responds the same way as it does in the oxyntic gland and small bowel mucosa.

Key words: *polyamines, colonic mucosa, rats, GI hormones, ODC*

INTRODUCTION

The regulation of gastrointestinal mucosal growth depends on a variety of hormonal and luminal factors called into play by the presence of food within the digestive tract. Three of the more important peptides that regulate mucosal growth are the hormones gastrin and secretin and the growth factor EGF (1). Gastrin stimulates growth of the gastric oxyntic gland, small bowel and colonic mucosa. Secretin inhibits these trophic actions of gastrin. EGH also stimulates gastrointestinal mucosal growth, and in the oxyntic gland mucosa the effect of EGF is not inhibited by secretin (2).

In the oxyntic gland and duodenal mucosa the tropic action of gastrin is prevented by inhibition of ornithine decarboxylase (ODC; EC 4.1.1.17) with DFMO (α-difluoromethylornithine) even though gastrin does not increase ODC activity as measured by the standard $^{14}CO_2$ collection (3). Using a highly specific polyclonal antibody we demonstrated that gastrin increased ODC levels only in the mucous neck cells of the oxyntic gland mucosa (4) and the crypt cells of the small intestinal mucosa (5). We concluded that since these proliferative cells made up only small fractions of the totals present that these increases in ODC activity were masked or diluted by other cells and were undetectable by the standard assay employed. EGF, on the hand, is a potent stimulator of ODC. Feldman et al. (6) found increased ODC levels in stomach, duodenum and colon after EGF administration to neonatal mice. Fitzpatrick et al. (7) found increased ODC activity in mucosa from all regions of the adult rat small intestine 4 h following a single injection of EGF.

The role of ODC stimulation in the growth of colonic mucosa is less clear. DFMO did not inhibit the growth of colonic mucosa in response to gastrin in the same rats in which it prevented the stimulation of gastric and duodenal mucosal growth (3). Similarly DFMO had little or no effect on colonic growth stimulated by refeeding fasted rats or when administered to normally fed animals (8). In these same animals DFMO again inhibited growth of mucosa from more orad portions of the digestive tract. These data suggest that polyamines may be supplied to the proliferative cells of the colon from a source other than endogenous ODC. In the current study we examined the regulation of colonic ODC by the trophic peptides gastrin and EGF and the inhibitory hormone secretin. Our results show that these regulatory peptides have the same effects on colonic ODC as they do on mucosal growth in the colon and on ODC in other gastrointestinal mucosae.

MATERIAL AND METHODS

Animals:

Adult male Sprague-Dawley rats weighing 140—160 g were used for all studies. Animals were housed individually in open-meshed, wire-bottomed, raised cages in a room with a 12 h light-dark cycle. Water and Purina Rat Chow were available ad libitum unless otherwise specified. Rats were obtained from Timco Breeding Laboratories (Houston, TX).

All rats were fasted 48 h with access to water. After fasting the rats were divided into the following groups: controls were injected with saline intraperitoneally; others were refed; the third group was injected with EGF ($150 \mu g \cdot kg^{-1}$); and the fourth group was injected with pentagastrin ($250 \mu g \cdot kg^{-1}$). All rats were killed 4 h later and the large intestines collected for measurement of ODC. There were 6 animals in each group.

In a second experiment rats were fasted as before and divided into groups as follows: controls were injected with saline; others were injected with EGF ($150 \mu g \cdot kg^{-1}$); a third group was injected

with pentagastrin (250 $\mu g \cdot kg^{-1}$); the fourth group was injected with the same dose of EGF plus secretin (25 $\mu g \cdot kg^{-1}$); the fifith group was injected with the same dose of pentagastrin plus secretin (25 $\mu g \cdot kg^{-1}$); and the final group was injected with secretin alone. All rats were killed 4 h later and the large intestines collected for measurement of ODC. Each group contained six rats. Doses of peptides were those previously shown to stimulate or, in the case of secretin, inhibit mucosal growth in colonic mucosa (1).

In a third study rats were fasted as before and injected intraperitoneally with pentagastrin, EGF or refed. Animals injected with gastrin or EGF were killed either 2 or 4 h later, and refed rats were killed 2 h later.

Biochemical assay of ODC

Rats were killed between 10 a. m. and 12 noon by exsanguination following ether anesthesia. A midline abdominal incision was made to expose the large intestine. We then collected the following samples: the entire cecum, a 3 cm segment of proximal colon beginning 1 cm from the ileal-cecal junction, and the distal 3 cm segment of colon. The segments were cut open and rinsed in ice-cold saline. The mucosa was scraped from the underlying smooth muscle with a glass microscope slide against a glass plate over ice. Mucosa was divided into two portions and weighed. One portion was used to measure ODC, the other to determine protein content.

The activity of the enzyme ornithine decarboxylase was assayed by a radiometric technique in which the amount of $^{14}CO_2$ liberated from L-[1-^{14}C] ornithine (51.3 mCi/mmol, New England Nuclear, Boston, MA) was determined (9). Tissues were collected as above and placed in 1.0 ml, pH 7.4, 67 mM sodium-potassium phosphate buffer containing 0.02% lauryl ether, 0.5 mM NaF, 0.1 mM pyridoxal phosphate, 10 μM EDTA, and 2 mM dithiothreitol (DTT). ODC activity is dependent on pyridoxal phosphate, and DTT stabilizes the enzymatic activity (10). The mucosa was homogenized, sonicated, and then centrifuged at 30,000 g at 4°C for 30 min. A 200 μl aliquot of the supernatant was incubated in a stoppered test tube in the presence of 2.5 nM L-[^{14}C] ornithine for 15 min at 37°C. The liberated $^{14}CO_2$ from the decarboxylation of ornithine was trapped on a piece of filter paper impregnated with 20 μl of 2.0 N NaOH, which was suspended in a center well above the reaction mixture. The reaction was stopped by the addition of trichloracetic acid to a final concentration of 10%. The $^{14}CO_2$ trapped in the filter paper was measured by liquid scintillation spectroscopy at a counting efficiency of 79%. Aliquots of the 30,000 g supernate were assayed for total protein, using the method described by Bradford (11). Enzymatic activity was expressed as pmol $^{14}CO_2$/h/mg of protein.

Immunocytochemical determination of ODC:

Rats were killed as described above and segments of proximal colon were sectioned so that four samples were available for staining. Samples were fixed in B-5 solution (containing 12 g $HgCl_2$ plus 2.5 g NaHAc added to 200 ml H_2O containing 4% formaldehyde) for 4 h at room temperature. Specimens were transferred to 70% ethanol until processing.

Paraffin-embedded sections were stained for ODC using immunoperoxidase. Before staining slides were deparaffinized with xylene and ethanol followed by treatment with iodine and sodium thiosulfate solution. The Vectastain ABC kit (Vector Labs, Burlingame, CA) was used for visualization of ODC (12, 13). Tissue sections were incubated at room temperature under the following conditions: 50 mM tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl), pH 7.4 containing 0.9% NaCl [Tris-buffered saline (TBS)] for 5 min, methanol containing 10% H_2O_2 for 10 min, sequentially in avidin solution and biotin solution for 15 min each with intermittent 5-min washes with TBS, 1% normal goat serum in TBS for 30 min, ODC antiserum at the indicated

dilutions for 30 min, and then avidin-biotinylated horseradish peroxidase complex for 30 min. The peroxidase reaction was initiated by the addition of 0.5% diaminobenzidine HCl, 0,4% $NiCl_2$, 0.01% H_2O_2, and terminated by washing with TBS. Slides were counterstained with methyl green. Controls for the specificity of the staining reaction were run by omitting the ODC antiserum or by substituting preimmune serum or neutralized ODC antiserum for the actual ODC antiserum.

ODC antibody was elicited in a rabbit by injection of ODC purified from cultured murine RAW 264 cells (14). Purification and characterization of the antigen as well as the specificity of the antibody have been described in detail in our previous publications (4, 5).

RESULTS

As shown in *Table 1* refeeding the fasted rats or injection of EGF increased ODC levels in all three parts of the large intestine. Basal and stimulated activities of the enzyme were much higher in cecal mucosa than in either the proximal or distal colon. Pentagastrin also increased ODC in the cecum to levels comparable to those stimulated by refeeding and EGF. Pentagastrin had no significant effect on enzyme activities in mucosa from either of the other regions of the bowel.

Table 1. Ornithine decarboxylase activity (pmol-mg protein^{-1} h^{-1}) in cecal and colonic mucosa from 48 h fasted rats killed 4 h following saline injection, refeeding, EGF (150 $\mu g \cdot kg^{-1}$) injection, or pentagastrin (250 $\mu g \cdot kg^{-1}$) injection. Means and SEM, n = 6.

	Group			
	Saline	Refed	EGF	Pentagastrin
Cecum	3.03 ± 1.38	66.3 ± 25.6*	67.5 ± 14.0*	54.4 ± 27.0*
Proximal Colon	0.94 ± 0.15	6.81 ± 2.99*	19.4 ± 6.61*	1.18 ± 0.30
Distal Colon	2.29 ± 0.66	13.5 ± 6.18*	26.2 ± 11.8*	1.49 ± 0.24

*, $p < 0.05$ compared to saline

In cecal mucosa secretin totally inhibited the increase in ODC stimulated by pentagastrin *(Fig. 1)*. On the other hand, secretin had no effect on the stimulation of ODC in response to EGF. Secretin given alone did not alter ODC activity (data not shown). *Figure 2* demonstrates that EGF but not pentagastrin increased ODC levels in proximal colonic mucosa. As was the case with cecal mucosa, secretin had no significant effect when administered with EGF.

Since gastrin is a known trophic hormone for colonic mucosa and since we were unable to show increased ODC levels in proximal or distal colon following pentagastrin, we employed an immunocytochemical technique to determine whether gastrin actually does increase ODC activity. *Figure 3* demonstrates the localization of ODC in control (fasted), refed, pentagastrin--treated and EGF-treated rats. Antibody dilution was 1:300 which was the

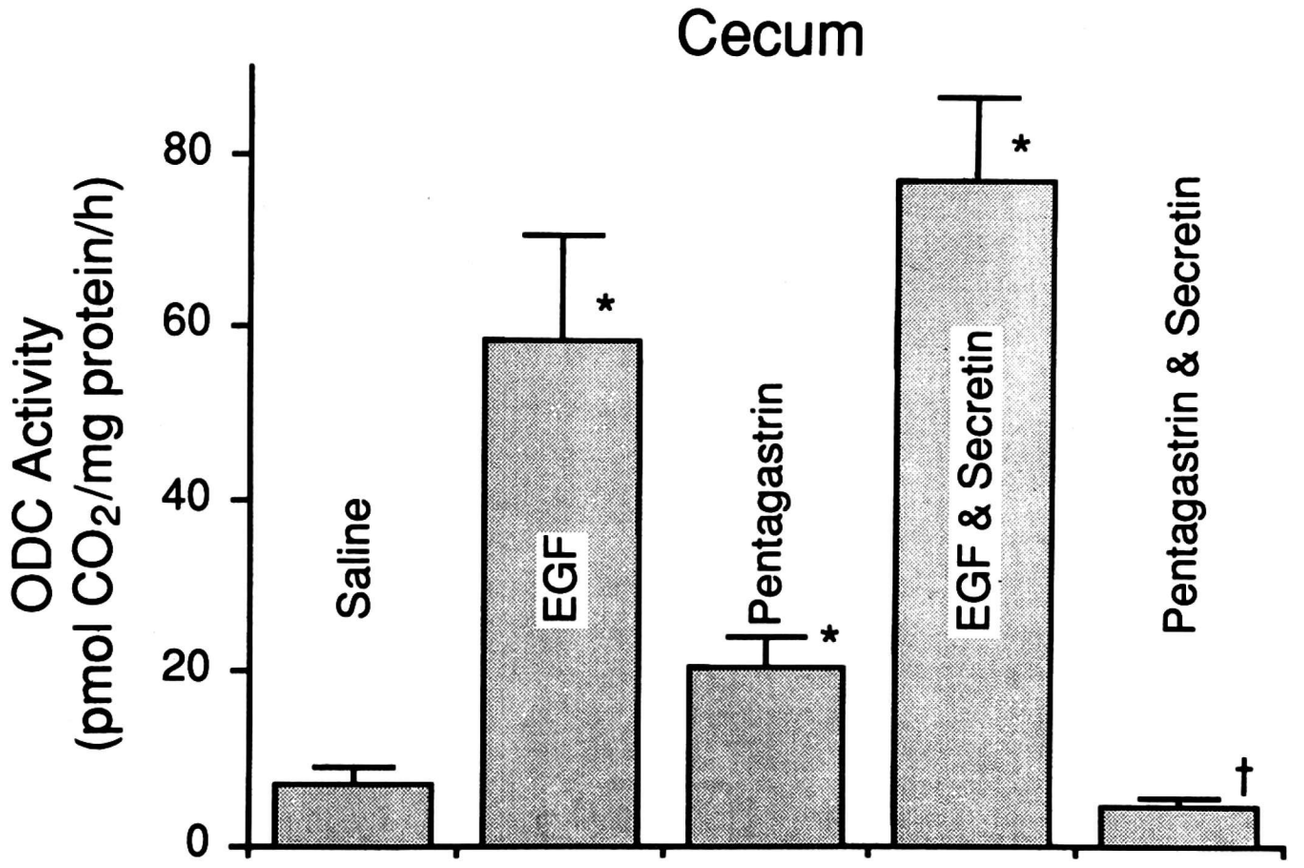

Fig. 1. ODC activity in cecal mucosa from 48 h fasted rats injected with saline, EGF (150 $\mu g \cdot kg^{-1}$) or pentagastrin plus secretin. Rats were killed 4 h later. Means and SEM of data from 6 rats. *, $p < 0.05$ compared to saline. †, $p < 0.05$ compared to pentagastrin.

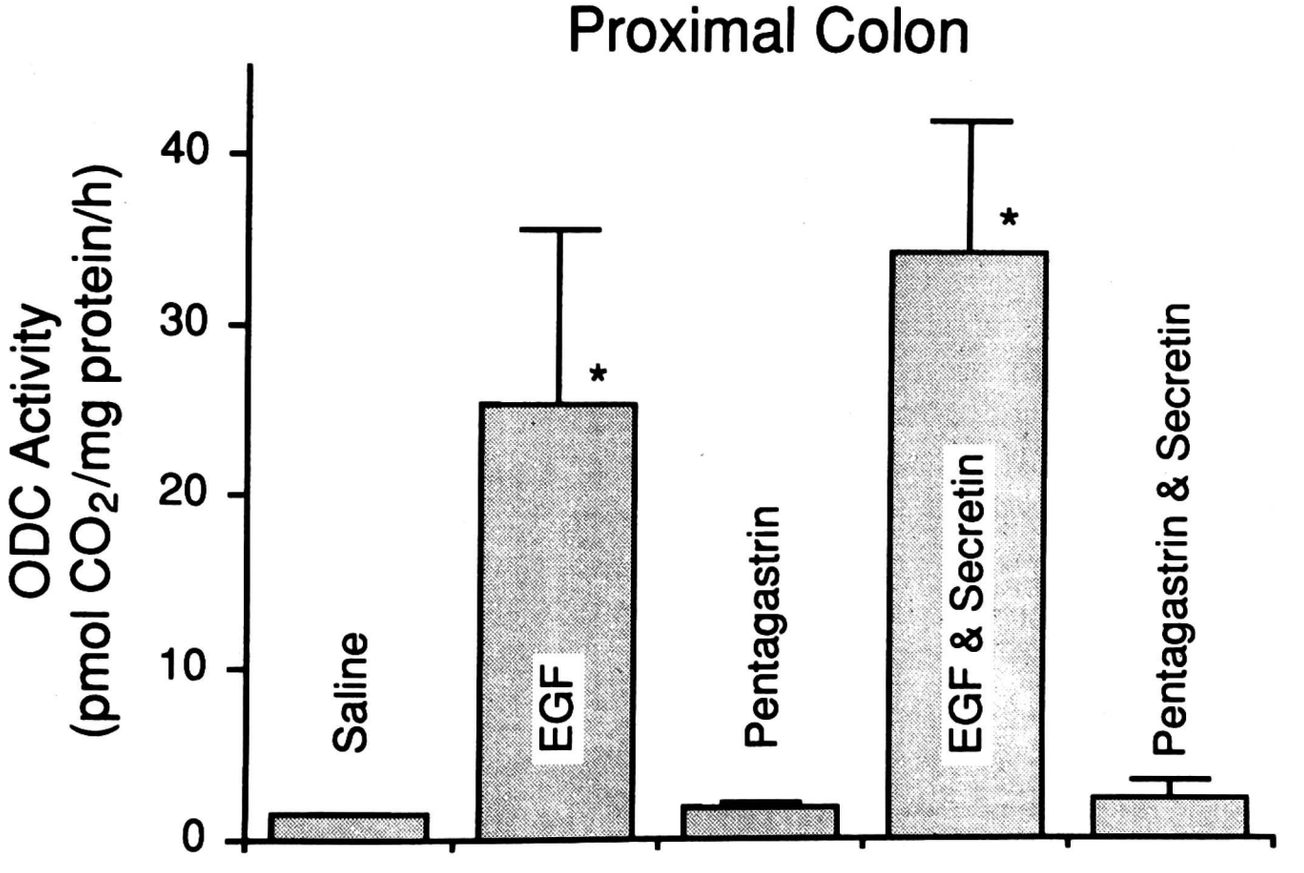

Fig. 2. ODC activity in mucosa of the proximal colon from rats described in Fig. 1. *, $p < 0.05$ compared to saline.

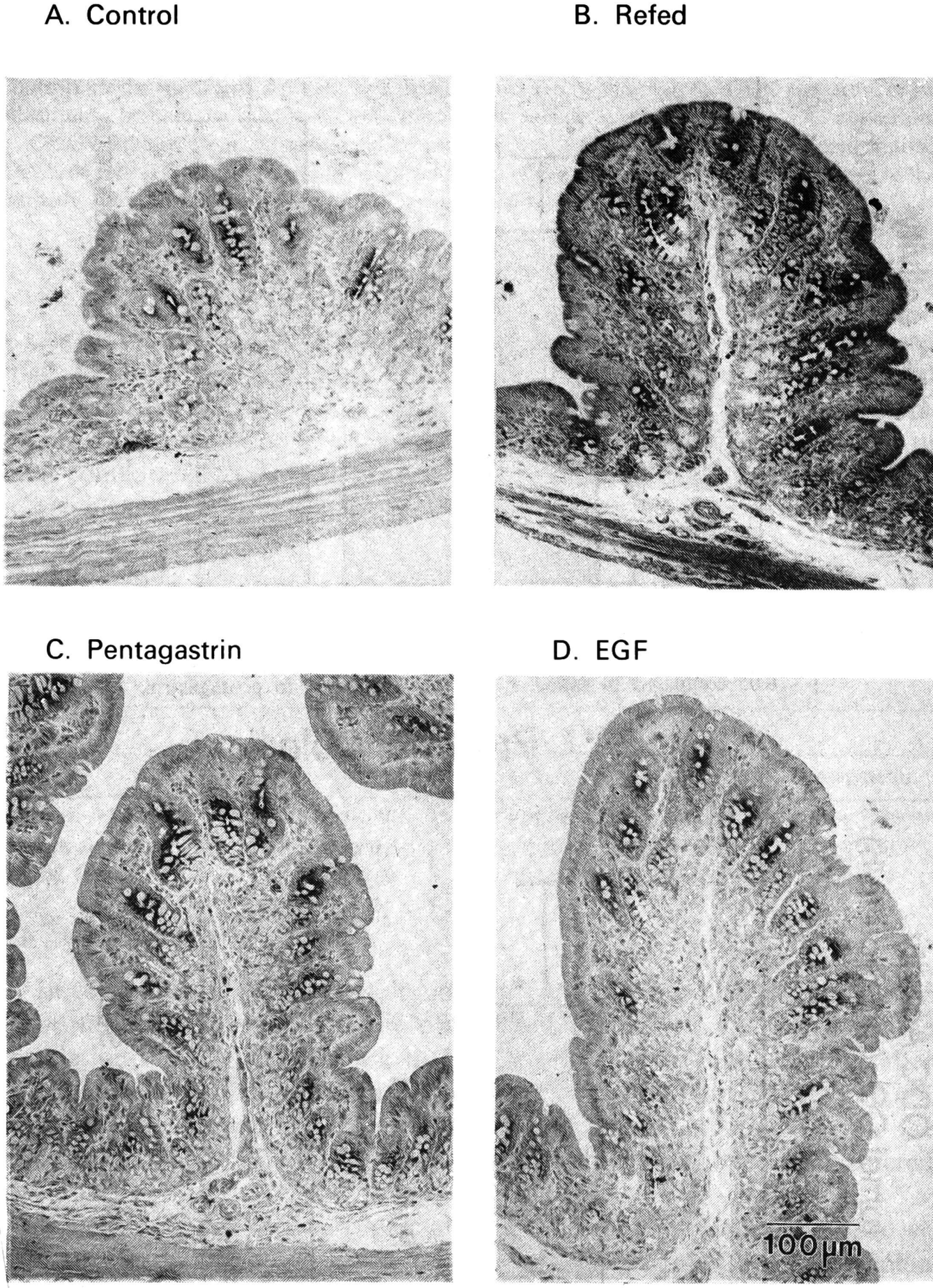

Fig. 3. Immunoperoxidase localization of ornithine decarboxylase in proximal colonic mucosa. All rats were fasted 48 h and then either refed or injected with different agents. Magnification x 120. Antibody dilution 1:300. A: Saline injected control, B: Refed for 2 h, C: 2 h following pentagastrin injection (250 $\mu g \cdot kg^{-1}$), D: 2 h following EGF injection (150$\mu g \cdot kg^{-1}$).

	Dilution of Antiserum			
	1:300	1:500	1:700	1:900
Fast	+	−	−	−
Gastrin - 2h	+	+	−	−
Gastrin - 4h	+	+	−	−
EGF - 2h	+	+	+	−
EGF - 4h	+	+	+	−
Refed - 2h	+	+	−	−

Fig. 4. Presence (+) or absence (−) of ODC staining in proximal colonic mucosa at various antiserum dilutions. Rats were fasted 48 h and killed at times shown after pentagastrin(250 $\mu g \cdot kg^{-1}$) or after refeeding for 2 h.

dilution required in order to visualize enzyme staining in control animals. In all groups of rats cells in the crypt region are heavily stained. In addition, refed rats show enzyme staining in the supranuclear regions of surface epithelial cells. With further dilution of antibody to 1:500 staining disappeared in sections from fasted rats *(Fig. 4.)* Staining was still visible, however, in sections from rats killed 2 or 4 h following injection with pentagastrin or EGF and in refed rats. At an antibody dilution of 1:700 staining was no longer visible in sections from pentagastrin treated rats. At this dilution, however, ODC was readily visible in sections from rats injected with EGF. These results demonstrate that pentagastrin does increase the amount of ODC in mucosa from the proximal colon even though the increase is undetectable by conventional biochemical assay.

DISCUSSION

The current experiments have demonstrated for the first time that 1) EGF increases ODC activity in muscosa from all regions of the large intestine; 2) gastrin causes large increases in ODC levels in the cecum and immunocytochemically detectable increases in mucosa from the proximal colon; 3) secretin inhibits the stimulation of ODC by gastrin but not EGF; 4) trophic

hormones increase ODC in the proliferative crypt of colonic mucosa; and 5) refeeding increases ODC in colonic surface epithelial cells as well as crypt cells.

From these finding it is obvious that the regulation of ODC in colonic mucosa is similar to that in gastric and small bowel mucosa. The trophic peptides EGF and gastrin stimulated ODC in the proliferative crypt cells. In the small intestine gastrin and EGF also increased ODC in crypt cells (9). Although most ODC is present in the villous enterocytes, gastrin had no effect on enzyme levels in these cells. Instead, luminal nutrients such as glycine specifically increased ODC in villous cells (9). The same situation appears to exist in colonic mucosa, as well as crypt cells. Drawing from our knowledge of the regulation of ODC in the small intestine, it seems logical to conclude that the effects of refeeding on ODC of colonic mucosa are due to gastrin acting on crypt cells and luminal agnts acting on surface cells. However, ODC increased within 2 h after refeeding which is a short time interval for newly ingested food to reach the colon. In oxyntic gland mucosa gastrin only increases ODC in the proliferative mucous neck cells (4).

The effects of secretin on ODC of large bowel mucosa were also identical to its effects on growth in other mucosa. In mucosa from the oxyntic gland, small intestine and colon, secretin inhibits the trophic action of gastrin and has no effects of its own (1). In a comparison of the trophic effects of pentagastrin and EGF, while secretin inhibited gastrin stimulated DNA synthesis in oxyntic gland mucosa it had no effect on that stimulated by EGF (2). Those data are also similar to the current findings. Since the inhibition of gastrin by secretin is due to a non-competitive interference with the binding of gastrin to its receptor (15), the current results are the predicted ones.

The findings that colonic mucosal growth is not as susceptible to inhibition by DFMO as is growth of mucosa from other regions of the gastrointestinal tract (3, 8) implies the existence of an alternate source of polyamines to support cell division in colonic mucosa. In earlier studies we have shown that enterocytes contain specific polyamine transport mechanisms and that they accumulate these substances (16). In addition luminal polyamines stimulate the growth of the intestinal mucosa (17). Osborne and Seidel (18) have obtained data supporting the hypothesis that mucosal hypertrophy in response to colonic obstruction is modulated by luminal polyamines derived from microflora. The bacteria present in the colon synthesize large amounts of polyamines. Therefore, polyamines of bacterial origin are available for use by the proliferative cells of the colonic mucosa. This would explain the relative independence of colonic mucosal growth from polyamines synthesized by ODC present in the dividing cells and why DFMO does not inhibit mucosal growth in the colon as readily as in other tissues. From the current studies, however, it is obvious that the normal peptide regulators of mucosal growth affect colonic ODC activity in the same manner as they do in other tissues.

Acknowledgement: DFMO (α-difluoromethylornithine) was the kind gift of the Merrell Dow Research Institute, Cincinnati, Ohio. This study was supported by grant DK16505 from the National Institutes of Health.

REFERENCES

1. Johnson LR. Regulation of gastrointestinal mucosal growth. *Physiol Rev* 1988; 68: 456—502.
2. Johnson LR, Guthrie PD. Stimulation of rat oxyntic gland mucosal growth by epidermal growth by epidermal growth factor. *Am J Physiol* 1980; 238: G45—G49.
3. Seidel ER, Tabata K, Dembinski AB, Johnson LR. Attenuation of the trophic response to gastrin after inhibition of ornithine decarboxylase. *Am J Physiol* 1985; 149: G16—20.
4. Johnson LR, Tseng C-C, Tipnis UR, Haddox MK. Gastric mucosal ornithine decarboxylase: localization and stimulation by gastrin. *Am J Physiol* 1988; 255: G304—G312.
5. Johnson LR, Tseng C-C, Wang P, Tipinis UR, Haddox MK. Mucosal ornithine decarboxylase in the small intestine: localization and stimulation. *Am J Physiol* 1989; 256: G24—G30.
6. Feldman EJ, Aures D, Grossman MI. Epidermal growth factor stimulates ornithine decarboxylase activity in the digestive tract of mouse. *Proc Soc Exp Biol Med* 1978; 159: 400—402.
7. Fitzpatrick LR, Wang P, Johnson LR. Effect of epidermal growth factor on polyamine-synthesizing enzymes in rat enterocytes. *Am J Physiol* 1987; 252: G209—G214.
8. Tabata K, Johnson LR. Ornithine decarboxylase and mucosal growth in response to feeding. *Am J Physiol* 1986; 251: G270—G274.
9. Pegg AE, McCann PP. Polyamine metabolism and function. *Am J Physiol* 1986; 143: C212—C221.
10. Russell DH, Snyder SH. Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo and various tumors. *Proc Natl Acad Sci* USA 1968; 60: 1420—1427.
11. Bradford MA. A rapid and sensitive method for the quantitation of microgram amounts of protein using the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248—254.
12. Hsu SM, Raine L, Fanger H. Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: A comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981; 29: 577—580.
13. Quian Z-U, Tsai Y-H, Steinberger A, Lu M, Greenfield ARL, Haddox MK. Localization of ornithine decarboxylase in rat testicular cells and epididymal spermatozoa. *Biol Reprod* 1985; 33: 1189—1195.
14. Greenfield ARL, Taffet SM, Haddox MK. Immunocytochemical localization of ornithine decarboxylase in cultured murine cells. *Cell Tissue Res* 1986; 243: 33—40.
15. Takeuchi K, Speir GR, Johnson LR. Mucosal gastrin receptor. IV. Binding specificity. *Am J Physiol* 1980; 239: G395—G399.
16. Kumagai J, Johnson LR. Characteristics of putrescine uptake in isolated rat enterocytes. *Am J Physiol* 1988; 254: G81—G86.
17. Seidel ER, Haddox MK, Johnson LR. Illeal mucosal growth during infusion of ethylamine or putrescine. *Am J Physiol* 1985; 149: G234—G438.
18. Osborne DL, Seidel ER. Microflora-derived polyamines modulate obstruction-induced colonic mucosal hypertrophy. *Am J Physiol* 1989; 256: G1049—G1057.

Received: September 5, 1991
Accepted: September 30, 1991

Author's address: Dr. Leonard R. Johnson Department of Physiology and Biophysics, University of Tennessee Nash Research Building 894 Union Avenue Memphis, Tennessee 38163, USA