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Prostaglandin-Mediated Trophic Effects on the Rat Duodenum: The Role of Polyamines[1] (42798)

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Abstract. Ornithine decarboxylase (ODC) is the initial rate-limiting enzyme in polyamine biosynthesis. The relationship between ODC and polyamines and the trophic effect of prostaglandin in the rat gastrointestinal tract are unknown. In these studies we determined whether inhibition of ODC activity and subsequent polyamine biosynthesis with the specific enzyme inhibitor difluoromethyornithine (DFMO) would attenuate prostaglandin-mediated trophic effects in the rat duodenum. Significant increases in duodenal mucosal wet weight, RNA, DNA, and protein were found following treatment with 16,16-dimethyl-PGE_2 (1 mg/kg) for 1 week. This trophic response was significantly reduced ($P < 0.01$) in the duodenum of rats treated concomitantly with DFMO. In addition, this prostenoid significantly increased (4×) duodenal ODC levels, 2 hr following acute administration. These results suggest that polyamines are required for the prostaglandin-stimulated growth of the rat duodenal mucosa.

It has become increasingly clear that the polyamines are required for cell growth (1, 2). A number of factors (3, 4) having trophic activity on gastrointestinal mucosa are also known to induce small intestinal ornithine decarboxylase (ODC) activity, the initial rate-limiting enzyme in polyamine biosynthesis. For example, we have recently shown that intraperitoneal EGF administration stimulates ODC activity in isolated rat enterocytes (4).

Chronic oral administration of large doses of PGE_2 or its methyl analogs can produce pronounced trophic effects on the rat stomach and small intestine (5). In addition, ODC activity increases in various tissues shortly after prostaglandin administration (6, 7). The relationship among ODC, polyamines, and the trophic effects of prostaglandins in the rat gastrointestinal tract is unknown. In the current study, we have examined the relationship between polyamine biosynthesis and 16,16-dimethyl-PGE_2-stimulated growth of rat duodenal mucosa.

The goal of this study was threefold: (i) To determine whether 16,16-dimethyl-PGE_2 administration increases ODC activity in the rat duodenum, (ii) to determine the magnitude of the prostaglandin-mediated trophic effect on the duodenal mucosa, and (iii) to determine whether inhibition of ODC activity and subsequent polyamine biosynthesis with difluoromethylornithine (DFMO), a specific inhibitor of ODC, altered the observed trophic effects.

Materials and Methods. *Animals.* Male Sprague–Dawley rats (Harlan Sprague Dawley, Houston, TX) weighing between 150 and 175 g were used in these studies. Standard rat pellets and water were provided *ad libitum.* Animals were given 16,16-dimethyl-PGE_2 (1 mg/kg) or a similar volume of isotonic saline intragastrically (ig) twice daily for 1 week. Rats were sacrificed 18 hr after the final dose. For acute studies, animals were fasted for 24 hr and then killed at varying times between 0.5 and 4 hr after ig dosing with the prostaglandin or saline. DFMO was administered to rats in chronic studies as a 2% solution in the drinking water.

Procedures. In the acute studies, a 5-cm segment of the duodenum (1 cm distal to the

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pylorus) was removed and retained for assay of ODC activity. The intestinal segment was rinsed in 0.9% saline and blotted. The mucosa was removed by scraping with a glass slide over ice. After homogenization, sonication (15 sec), and centrifugation (30,000*g*, 30 min), the supernatants were used for ODC determinations.

ODC activity was measured by the liberation of $^{14}CO_2$ from L-[1-^{14}C]ornithine (New England Nuclear, Boston, MA), as described previously (3, 4). Enzyme activity is expressed as picomoles per hour per milligram protein. Quantitative protein determination was by the Bradford (8) method.

Similarly, in chronic (1-week) studies, a 5-cm segment of duodenal mucosa was used for RNA, DNA, protein, ODC, and polyamine determinations. RNA, DNA, and protein contents were analyzed by methods described previously (9). ODC activity was measured as described above and polyamines (putrescine, spermidine, and spermine) were determined by high-performance liquid chromatography (3). RNA and DNA contents are expressed as micrograms per tissue, protein as milligrams per tissue, and polyamines as nanomoles per milligram protein.

Statistics. All results are given as means ± SE. Significance was established by analysis of variance and the level of significance by Duncan's multiple range test in chronic (1-week) studies. For acute studies, statistical significance was determined by two-tailed *t* test for unpaired data.

Results. Acute intragastric administration of 16,16-dimethyl-PGE_2 significantly increased ODC activity by 1 hr (Fig. 1). At this time activity was 230% of control. ODC activity peaked by 2 hr at 350% of control levels. By 4 hr activity had declined significantly to a value that was not statistically different from control levels.

Intragastric administration of 16,16-dimethyl-PGE_2 (1 mg/kg) twice daily for 1 week caused a significant increase in ODC activity in the rat duodenum (Table I). Intragastric administration of DFMO (2% in drinking water) led to the expected reductions in ODC activity. In addition (Table I) DFMO treatment for 1 week led to reductions in putrescine and particularly spermidine content in the rat duodenal mucosa. A paradoxical increase in duodenal spermine content, in DFMO-treated rats, as described by other investigators (1, 10) was also observed. Interestingly, no significant increases in the polyamine contents in the duodenal mucosa of prostaglandin-treated animals were observed in this study.

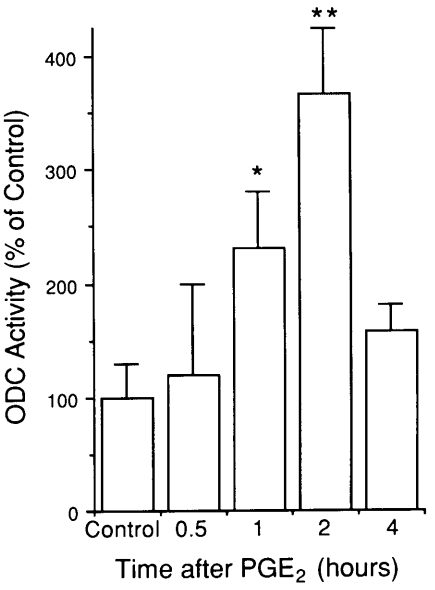

FIG. 1. The effect of intragastric 16,16-dimethyl-PGE_2 administration on ODC activity shown as percentage of control in rat duodenal mucosa at different time points after prostaglandin injection. Means ± SE of four measurements. $^*P < 0.05$; $^{**}P < 0.01$ compared to control.

There was also a significant increase in duodenal mucosal wet weight, as well as RNA, DNA, and protein content (Fig. 2), following chronic prostaglandin treatment. The increases in these various trophic indices were significantly reduced ($P < 0.01$) by DFMO.

Discussion. Chronic administration of prostaglandins is known to induce trophic changes in the rat gastrointestinal mucosa (5, 11). These effects occur throughout the length of the gastrointestinal tract, including the large intestine (5). Johansson *et al.* (5) found that the administration of 15,15-dimethyl-PGE_2 or PGE_2 to rats for 14 weeks caused significant trophic effects in the stomach, small intestine, and colon. These effects could not be explained by elevation of serum

TABLE I. EFFECT OF PROSTAGLANDIN + DFMO ON DUODENAL ODC ACTIVITY AND POLYAMINE LEVELS

	ODC activity (pmole/mg protein/hr) CO_2	Polyamine contents (pmole/mg protein)		
		Putrescine	Spermine	Spermine
Saline	1.5 ± 0.4	0.9 ± 0.4	2.2 ± 0.2	7.9 ± 0.8
Saline + DFMO	0.8 ± 0.4	0.3 ± 0.2	3.7 ± 0.7*	2.8 ± 0.5**
16,16-DMPGE$_2$	4.4 ± 1.0*	0.5 ± 0.1	1.3 ± 0.4	5.8 ± 0.9
16,16-DMPGE$_2$ + DFMO	0.4 ± 0.1**	0.4 ± 0.2	2.3 ± 0.3	1.8 ± 0.2**

Note. 16,16-DMPGE$_2$ was administered twice daily (1 mg/kg intragastrically) for 7 days. Values are means ± SE. N = 4–6 per group.

* $P < 0.05$ vs appropriate control.

** $P < 0.01$ vs appropriate control.

gastrin levels, a trophic hormone for the gastrointestinal mucosa, but were attributed to stimulation of other local growth factors by the prostanoid. Following the finding that acute PGE$_2$ increased duodenal mucosal ODC activity (Fig. 1), we hypothesized that increased cellular polyamines, formed via the rate-limiting enzyme ODC, might be required factors for the trophic effects observed following chronic prostaglandin administration. In order to assess this possibility, we examined the effect of administering a high dose of prostaglandin for 1 week, in the presence or absence of DFMO, on the rat duodenal mucosa.

As illustrated in Fig. 2, prostanoid administration (1 mg/kg) twice daily for 1 week increased duodenal mucosal wet weight. Similarly, pronounced increases in RNA, DNA, and protein content were also observed. Chronic prostanoid treatment also produced a statistically significant increase in duodenal ODC activity (Table I), but was not accompanied by a corresponding increase in the steady-state cellular levels of the polyamines. Concurrent administration of the specific inhibitor DFMO resulted in mucosal atrophy of the duodenum. Trophic indices were significantly reduced by DFMO treatment (Table I). In addition, concurrent administration of DFMO reduced tissue polyamine levels, particularly spermidine (Table I).

These results suggest that polyamines are required for the trophic effect of 16,16-dimethyl-PGE$_2$ on the rat duodenal mucosa. The reduction of ODC activity, as well as putrescine and spermidine contents, in rats dosed with prostaglandin plus DFMO is accompanied by significant reduction in various trophic indices. Similar results were reported by Seidel *et al.* (12), who found that

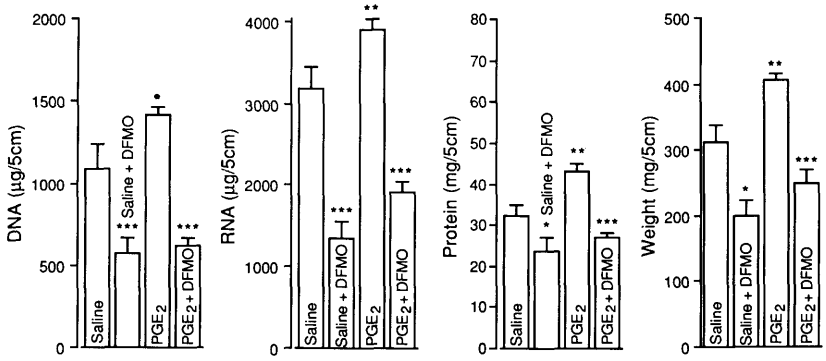

FIG. 2. Changes in duodenal mucosal RNA, DNA, protein, and wet weight from rats described in Fig. 2. *$P < 0.05$ compared to saline. **$P < 0.01$ compared to saline. ***$P < 0.01$ compared to the appropriate control (−DFMO).

concurrent treatment with DFMO completely inhibited the trophic response to pentagastrin in the rat oxyntic gland and duodenal mucosa. Dowling *et al.* (13) have reported that in the small intestine, unlike the pancreas, DFMO treatment markedly inhibited the adaptive hyperplasia induced by pancreatico-biliary diversion. The results of our study, as well as those of other investigators (12, 13), suggest that ODC and the polyamines may be preferentially associated with the trophic effects observed in the rat small intestine, following prostaglandin as well as during adaptation.

Although the results of this study suggest the need for intact polyamine synthesis for prostaglandin-mediated intestinal mucosal growth, we were unable to demonstrate significantly elevated levels of polyamines in the rat duodenum. It is possible that polyamine levels increased and then returned to normal at the time that tissue was collected for analysis (18 hr following the last prostanoid administration); however, ODC was significantly elevated at this time. More likely, prostaglandin administration may increase the rate of polyamine catabolism to a greater degree than biosynthesis following chronic dosing. Such a possibility has been suggested previously (12) with regard to the action of gastrin on the rat gastrointestinal tract.

This study is one of a few (15) that have investigated the effect of acute prostaglandin treatment on the induction of ODC in the gastrointestinal tract. Prostaglandins, however, have been shown to stimulate ODC activity in other rat tissues (6, 7). Recent results from our laboratory have shown induction of ODC activity in isolated rat enterocytes by trophic stimuli such as EGF (4) and refeeding (3). Other studies have demonstrated induction of enzyme activity in the duodenal mucosa by insulin (14). Thus, our finding that 16,16-dimethyl-PGE_2 induced duodenal mucosal ODC was not unexpected. Moreover, the peak induction of ODC activity (2 hr after administration) is similar to observations from our laboratory (3) and those of others (1, 10) for other stimuli. In addition, we found in this study a significant increase in duodenal SAMDC activity (polyamine biosynthetic enzyme) 4 hr after prostanoid administration (data not shown).

It is well recognized that the duodenal mucosa is maintained by epithelial cell proliferation in the crypts, that is, matched by cell loss from the villus tips into the duodenal lumen. The chronic administration of methyl-PGE_2 analogs is known to cause hyperplasia of the rat gastrointestinal epithelium by prolongation of the cell survival time (16). Whether ODC and/or the polyamines are involved in reducing cell loss was not assessed in this study. However, ODC has been proposed to play an important role in intestinal mucosal maturation (10).

In summary, we have shown significant induction of ODC activity in the rat duodenal mucosa (2 hr) after intragastric 16,16-dimethyl-PGE_2. Significant increases in various trophic indices were observed in the rat duodenum following chronic prostaglandin treatment. The trophic effect was not observed in rats treated concomitantly with DFMO. We conclude from these studies that the polyamines are required for the normal manifestation of prostaglandin-mediated trophic effects on the rat duodenal mucosa.

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