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STEREOLOGICAL ESTIMATION OF THE TOTAL VOLUME OF DUODENAL MUCOSA AND OF ENDOCRINE CELLS IN RATS TREATED WITH PROSTAGLANDIN E_2

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ABSTRACT

Stereological methods were applied on histological sections of 2 cm rat duodenum to determine the total volume of the mucosa, the total surface area of small intestinal epithelial lining and the total volume of endocrine cells. The effects of different oral doses of prostaglandin E_2 and a methyl analogue on the parameters above were estimated following 4 weeks of treatment. The total volume of serotonin-immunoreactive cells was increased by prostaglandins E_2, whereas the analogue reduced the total volume of somatostatin-immunoreactive cells compared to controls ($p<0.05$). The total mucosal volume and the total surface area of small intestinal epithelial lining were unaffected. Despite overestimation of endocrine cells in thick sections, the estimation of the profile area by image analysis disclosed that the changes in total volume induced by prostaglandins was most likely to be secondary to hyperplasia of endocine cells.

Key words: enterochromaffin cell, prostaglandin E_2, small intestine, somatostatin, stereology.

INTRODUCTION

Prostaglandin E_2 (PGE_2) induces trophic changes in the small intestinal mucosa of rat and man (Helander et al., 1985; Johansson et al., 1986; Henriksson et al., 1988; Uribe et al.,1988). In some of these investigations, high plasma levels of gastrointestinal peptides were observed (Helander et al.,1985; Johansson et al., 1986). Thus, the aim of the present investigation was to examine whether oral PGE_2 influences endocrine cells in the duodenum, which could further suggest potential interactions between biological active peptides and prostanoids. For this purpose, stereological methods were applied in histological sections of rat duodenal mucosa to quantify endocrine cells in terms of total volume.

MATERIAL AND METHODS

40 Sprague-Dawley rats were allocated to one of the following treatment groups: placebo (n=12), PGE_2 25 and 5000 µg/kg (n=6 in each group) or 15-R-15-Methylprostaglandin E_2 ($MePGE_2$) 5 and 50 µg/kg (n=8 in each group). All doses were given orally, twice a day for 4 weeks.

Tissue preparation and stainings

The animals were killed by cervical dislocation. A fixed length of two cm duodenum was exeised, opened along its longitudinal axis, weighed and fixed in buffered formaldehyde for 4 h. Thereafter one vertical biopsy specimen was cut at a random orientation following a blind rotation of the duodenal sample by the examiner using 2 razor blades mounted in parallel (Mattfeldt et al, 1985; Baddeley et al, 1986). The specimen was embedded in paraffin. Three µm thick sections were cut 50 µm apart and stained with haematoxylin-eosin or processed for immunochemistry. To visulize endocrine cells, rabbit polyclonal antibodies against human gastrin (cat. no A 568, 17-I, Dakopats, Denmark) was used at a dilution of 1:1200, monoclonal antibodies against synthetic serotonin in rat (MAS 055b clone Y C 5/45, Sera-Lab Ltd, Sussex, England) was used at a dilution of 1:1600 and rabbit antiserum against synthetic bovine somatostatin, kindly provided by Prof J H Rehfeld, Dept. Med Biochem, Copenhagen, Denmark was used at a dilution of 1:400 (Sternberger, 1979).

Stereological methods

The volume density (Vv) of the duodenal and intestinal mucosa were estimated at low power (x100, light microscopy). Fields of vision were projected on a desk where transparent stereological test system were superposed. The examined areas were chosen by systematic random sampling (Mathieu et al, 1981) following manual movements of the microscope stage. The (Vv) was determined by dividing the number of hits in the mucosa by the number of hits in the intestinal wall (Gundersen et al., 1981; 1987). Assuming that the specific density of intestine is approximately 1. The mucosal weight can be used as estimate of its volume (Mayhew, 1983). The total volume of the intestinal mucosa is: **Vv mucosa x weight**.

The volume density of endocrine cell populations

Endocrine cells (Ec-cells) was estimated following random movements of the microscope stage by the examiner until 100 cells had been screened (Michel et al., 1988). The total volume of endocrine cells in the duodenum is: **Vv Ec-cells x total volume of duodenal mucosa**.

The profile area of endocrine cells were determined by image analysis, to detect potential changes in cell size, induced by prostaglandins.

The surface density of small intestinal villous lining (Sv) was determined by using a cycloid system until 200 intersections on the luminal side of villi were recorded (Gundersen, 1988).

RESULTS

The total volume of serotonin-immunoreactive cells was increased in rats given PGE_2 ($p<0.05$, Fig1a) whereas $MePGE_2$ reduced the total volume of somatostatin cells ($p<0.05$, Fig1b). The total volume of gastrin-immunoreactive cells was not affected by treatments (Fig1c). PGE_2 did not affect the mucosal volume, the total surface area of villous lining and the profile area of endocrine cells (data not shown).

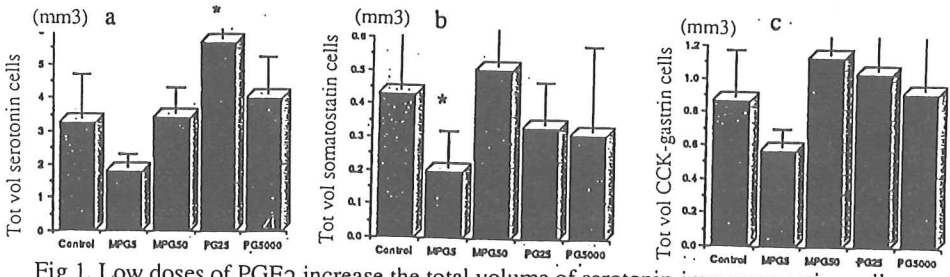

Fig 1. Low doses of PGE_2 increase the total volume of serotonin-immunoreactive cells (a), whereas $MePGE_2$ reduce the total volume of somatostatin cells (b). PGE_2 did not affect CCK-Gastrin cells (c). Values are find as mean ± SD.

DISCUSSION

In this study we applied stereological methods to quantitate total volume of endocrine cells and to detect potential trophic actions of prostaglandins. Our results disclose complex actions of prostaglandins on the endocrine cell system as shown by its stimulatory and inhibitory effects on duodenal endocrine cells. In addition, this study shows that PGE_2 selectively influence endocrine cells in the duodenum of the rat and that this change is not associated with an increased mucosal volume or an increased total surface area of villous lining. These findings suggest that prostaglandins and other factors may modulate the turnover of endocrine cells, which warrants further investigation.

The total volume of endocrine cells is overestimated in the used thick sections (Holmes, 1927; Gundersen, 1986). Due to the irregular shape of these cells we were not able to use correction formulas (Weibel, 1979). However, the estimation of the profile areas of endocrine cells by image analysis, it suggested that prostaglandins did not affect the size of these cells. This is indirect evidence suggesting that the changes in total volume might have been secondary to changes in the number of endocrine cells which is satisfactory for the purposes of this comparative study. At the present time, the optical disector should be the method of choice in quantitative studies of this kind (Gundersen, 1987).

In summary, we applied stereological methods in rat duodenal mucosa to determine total volume of endocrine cells and total surface area of small intestinal villous lining as well as the total volume of 2 cm duodenal mucosa. Oral E_2 prostaglandins selectively affect serotonin- and somatostatin-immunoreactive cells in rat duodenum.

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