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# EFFECT OF 16,16-DIMETHYL PROSTAGLANDIN $E_2$ ON MUCUS GLYCOPROTEIN BIOSYNTHESIS IN RAT STOMACH AND DUODENAL GLANDS

Thea Jentjens and Ger J. Strous
Department of Cell Biology, School of Medicine, University of Utrecht,
Nic. Beetsstraat 22, 3511 HG Utrecht, The Netherlands
(reprint requests to GS)

## ABSTRACT

The present study describes the effect of 16,16-dimethyl prostaglandin $E_2$ (16,16-dmPGE$_2$) on mucus glycoprotein biosynthesis in rat stomach and duodenal glands. After *in vivo* treatment with 16,16-dmPGE$_2$ (10 µg/kg subcutaneously) for 1 h, the incorporation rate of [$^3$H]galactose, [$^3$H]glucosamine, and [$^3$H]serine in the *ex vivo* vascularly perfused stomach was determined by light microscopic autoradiography.

As was previously found by us for the surface mucous cells in the fundus of 16,16-dmPGE$_2$-treated rats, the incorporation of [$^3$H]galactose and [$^3$H]glucosamine (indicative of mucus glycoprotein synthesis) in the isthmus was increased two- to fourfold. Small if any increases were detected in the mucous cells near the base of the glands of the fundus (neck cells), the mucous cells in the antrum and the mucous cells in the duodenal glands. Total protein synthesis as measured by [$^3$H]serine incorporation was not increased in any of these cells.

We conclude that 16,16-dmPGE$_2$ has different effects on mucus glycoprotein biosynthesis in various regions of the rat stomach. Increased biosynthesis in the fundus points to a role for mucus in the prostaglandin-induced protection of the gastric mucosa against injury.

## INTRODUCTION

Several prostaglandins inhibit the formation of macroscopically visible gastric lesions induced by harmful agents such as absolute ethanol (1) or aspirin (2). However, detailed microscopic studies revealed that protection of the gastric mucosa was not complete: 16,16-dimethyl prostaglandin $E_2$ (16,16-dmPGE$_2$) did not prevent ethanol-induced damage to the surface mucous cells (3-5). The mechanism or mechanisms of this so-called cytoprotection has not yet been elucidated.

In our investigations into the role of mucus in gastric protection by prostaglandin, we have studied the effect of 16,16-dmPGE$_2$ on mucus glycoprotein biosynthesis in the rat stomach. Using the isolated vascularly perfused rat stomach (6), which is an ideal system for the

study of the effects of drugs on mucus biosynthesis, we recently demonstrated that in the surface mucous cells of the fundus 16,16-dmPGE$_2$ increased [$^3$H]galactose and [$^3$H]glucosamine incorporation two- to fourfold, but had no effect on the incorporation of [$^3$H]serine (7). This observation proves that 16,16-dmPGE$_2$ stimulates mucus glycoprotein synthesis but has no stimulatory effect on the overall protein synthesis of the surface mucous cell.

We were intrigued by the fact that under extreme conditions prostaglandins appear to be unable to protect the surface mucous cells from damage. Consequently we have now extended our studies into the effect of 16,16-dmPGE$_2$ on the glycoprotein synthesis, to include the mucous cells located deeper in the gastric mucosa, i.e. the mucous cells in the isthmus and neck of the fundic glands. Part of the material obtained in our earlier studies (7) is used for comparison in this study. Since a difference between fundus and antrum in susceptibility for ethanol and aspirin has been reported (4,8), we also compared the effect of 16,16-dmPGE$_2$ on glycoprotein synthesis in fundus, antrum as well as in duodenal glands.

## MATERIALS AND METHODS

### Chemicals

Fluorocarbon FC-75 was obtained from the 3M Company (St. Paul, MN, USA). Bovine serum albumin and amino acids were from Sigma Chemical Co. (St. Louis, MO, USA). Radiochemicals were purchased from Amersham International plc (Amersham, UK). All other chemicals were of analytical grade and obtained from Merck (Darmstadt, FRG). 16,16-dmPGE$_2$ was kindly supplied by Dr. A. Robert and Dr. J. E. Pike (Upjohn Co., Kalamazoo, Mich.).

### Experimental animals

Male Wistar rats weighing 200-250 g were subjected to a strict fasting-feeding protocol to synchronize and precondition them. This consisted of a 22-h fasting period followed by free access to food for 2 h and another fasting period for 16 h. They had water *ad libitum*.

### Prostaglandin treatment

Rats received 10 µg/kg body weight of 16,16-dmPGE$_2$ (10 µg 16,16-dmPGE$_2$/ml saline) subcutaneously 1 h before starting the perfusion. Control animals received an equivalent volume of saline.

### Perfusion system

The vascular perfusion system as described by van Huis and Kramer (6) was used with a few minor modifications. Rats were anaesthetized by i.p. injection of pentobarbitone sodium (50 mg/kg body weight). After a 1-min perfusion with Tyrode's solution, perfusion with "perfusion medium" was initiated and maintained for 1 h. The perfusion medium consisted of Tyrode's solution containing 5% (w/v) bovine serum albumin and 10% (w/v) fluorocarbon FC-75, emulsified beforehand. Amino acids were added at plasma concentrations; 16,16-dmPGE$_2$ was added to a final concentration

of 0.05 µg/ml. During the perfusion, the medium was continuously oxygenated and thermostabilized at 37 °C. The pH of the medium was maintained at 7.4.

Incorporation of precursors and fixation

After 30 min perfusion, D-[1-$^{3}$H]galactose (22 Ci/mmol), D-[6-$^{3}$H]glucosamine(20 Ci/mmol), or L-[3-$^{3}$H]serine (29 Ci/mmol) was added to the perfusion medium at a final concentration of 10 µCi/ml. The perfusion was stopped after a labeling period of 30 min and the stomach removed from the perfusion system. The stomach was immediately cut open along the greater curvature and washed in 0.1 M phosphate buffer (pH 7.4). Cubes containing fundus and antrum of the stomach and the proximal part of the duodenum were fixed in 1% paraformaldehyde, 1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4).

Light microscopic autoradiography

The fixed tissue blocks were washed in 0.1 M phosphate buffer (pH 7.4), dehydrated in graded alcohol concentrations, and embedded in glycol-methacrylate. Five-micron sections were cut on a Sorvall Porter-Blum MT-1 microtome and mounted on glass slides precoated with 1% gelatine. The sections were then covered by Kodak AR stripping film (Eastman Kodak, Rochester N.Y.) and exposed at 4 °C. The exposure time was 14 days for galactose and glucosamine labeling, 6 days for serine labeling of the fundus and antrum, and 1 day for serine labeling of the duodenum. After developing in Kodak D19b, the autoradiographs were stained with periodic acid-Schiff/haemalum in order to identify the different cell types in the mucosa. To determine the amount of radioactive precursor incorporated per cell, the number of silver grains over areas of mucous cells in randomly chosen sections of the fundus, antrum and duodenum was counted. These scores were divided by the number of nuclei visible within the same area, resulting in the mean number of silver grains per cell. A typical analysis involved three or four animals. Four sections per stomach and in each section 50 cells per cell type were analyzed.

The different categories of cells in the fundus and antrum are represented schematically in Figure 1. In the fundus the silver grains were counted in the uppermost 5 cells at the top of the foveola, in the mucous cells of the isthmus and of the neck of the fundic gland. These areas were delineated according to Wattel and Geuze (9): the uppermost parietal cell marks the boundary between the foveola and the isthmus, the uppermost mucous neck cell the boundary between the isthmus and the neck, and the lowest mucous neck cell the boundary between the neck and the base of the fundic gland. In the antrum the silver grains over the cells at the top of the foveola, in the isthmus (bottom of the foveola) and of the base were counted. In the duodenum the silver grains over the cells of the duodenal glands (submucosal or Brunner's glands) were counted. The duodenal glands are found in the submucosa of the duodenum and consist of only one cell type.

The Wilcoxon test was used for the statistical evaluation, and the level of significance was set at $p < 0.05$.

## RESULTS

Stomach perfusion for 1 h did not affect the structure of the tissue and after periodic acid-Schiff/haemalum staining the different classes of mucous cells could easily be distinguished. In Figure 1 the different categories of mucous cells in the fundus and antrum are schematically represented.

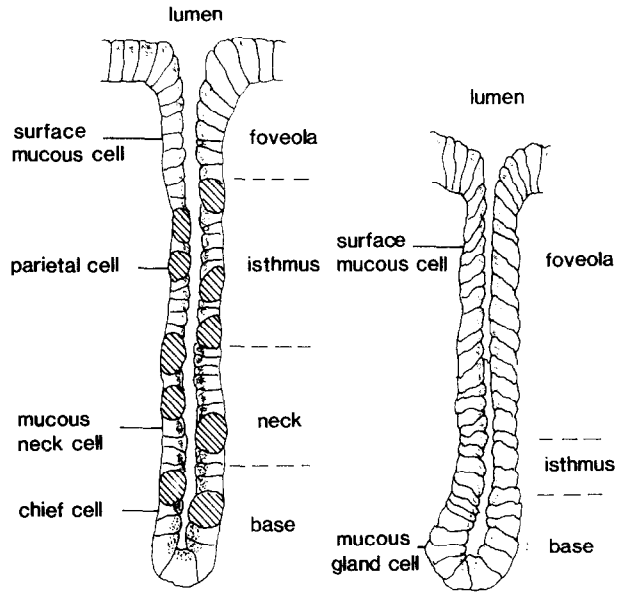


Fig. 1 Schematic representation of a fundic (left) and antral (right) gland.

Table 1 [$^3$H]Galactose incorporation in mucous cells of fundus, antrum and duodenal gland, after 1-h treatment with 16,16-dmPGE$_2$ (10 µg/kg). Control rats received saline. The mean number of silver grains per cell ± SEM is shown with the number of animals in parentheses.

| | Control | 16,16-dmPGE$_2$ |
|---|---|---|
| Fundic surface | 2.72 ± 0.96 (3) | 10.78 ± 0.66*(4) |
| Fundic isthmus | 1.76 ± 0.30 (3) | 6.69 ± 0.56*(4) |
| Fundic neck | 1.03 ± 0.26 (3) | 1.76 ± 0.36 (4) |
| Antral surface | 2.46 ± 0.75 (3) | 3.37 ± 1.12 (4) |
| Antral isthmus | 1.18 ± 0.36 (3) | 2.32 ± 0.83 (4) |
| Antral base | 0.79 ± 0.14 (3) | 0.75 ± 0.08 (4) |
| Duodenal glands | 3.81 ± 1.33 (3) | 5.60 ± 0.51 (3) |

* $p < 0.05$ compared with control (Wilcoxon test)

Table 2 [$^3$H]Glucosamine incorporation in mucous cells of fundus, antrum and duodenal gland, after 1-h treatment with 16,16-dmPGE$_2$ (10 µg/kg). Control rats received saline. The mean number of silver grains per cell ± SEM is shown with the number of animals in parentheses.

| | Control | 16,16-dmPGE$_2$ |
|---|---|---|
| Fundic surface | 3.13 ± 0.39 (3) | 5.68 ± 0.07*(3) |
| Fundic isthmus | 0.90 ± 0.15 (3) | 1.86 ± 0.16 (3) |
| Fundic neck | 0.37 ± 0.05 (3) | 0.54 ± 0.07 (3) |
| Antral surface | 0.74 ± 0.16 (3) | 1.06 ± 0.41 (3) |
| Antral isthmus | 0.69 ± 0.10 (3) | 0.80 ± 0.24 (3) |
| Antral base | 0.15 ± 0.05 (3) | 0.12 ± 0.01 (3) |
| Duodenal glands | 1.04 ± 0.32 (3) | 1.27 ± 0.39 (3) |

* $p < 0.05$ compared with control (Wilcoxon test)

Table 3 [$^3$H]Serine incorporation in mucous cells of fundus, antrum and duodenal gland, after 1-h treatment with 16,16-dmPGE$_2$ (10 µg/kg). Control rats received saline. The mean number of silver grains per cell ± SEM is shown with the number of animals in parentheses.

| | Control | 16,16-dmPGE$_2$ |
|---|---|---|
| Fundic surface | 7.29 ± 0.69 (3) | 7.59 ± 0.28 (3) |
| Fundic isthmus | 5.72 ± 0.31 (3) | 5.21 ± 0.58 (3) |
| Fundic neck | 6.25 ± 0.56 (3) | 5.89 ± 0.24 (3) |
| Antral surface | 7.25 ± 1.01 (3) | 7.34 ± 0.91 (3) |
| Antral isthmus | 6.74 ± 0.56 (3) | 6.22 ± 0.78 (3) |
| Antral base | 3.64 ± 0.77 (3) | 4.51 ± 0.60 (3) |
| Duodenal glands | 21.60 ± 1.20[a](3) | 19.14 ± 0.90[a](3) |

[a] determined on sections exposed for 1 day, but converted to 6 days

As can be seen from Table 1, the increase ($p < 0.05$) of [$^3$H]galactose incorporation in the mucous cells of the isthmus of the fundus was similar as the increase previously seen in the surface mucous cells after 16,16-dmPGE$_2$ treatment. The mucous neck cells of the fundus showed a much smaller increase (not statistically significant) in [$^3$H]galactose incorporation. In the antrum the incorporation of [$^3$H]galactose was slightly increased in the mucous cells of the surface and isthmus (not statistically significant); no increase was seen in the mucous cells of the antral base.

In the fundus, [$^3$H]glucosamine incorporation in the mucous cells of the isthmus was doubled, which is comparable to what was found previously in the surface mucous cells. In the antrum the incorporation

of this precursor was slightly increased in the surface mucous cells and in the mucous cells in the isthmus (not statistically significant); no increase was found in the mucous cells of the antral base. (Table 2).

No increase in the incorporation of [$^3$H]galactose and [$^3$H]glucosamine was seen in the mucous cells of the duodenal glands (Tables 1 and 2).

As can be seen from Table 3 none of the studied mucous cells showed an increase in the incorporation of [$^3$H]serine after prostaglandin treatment.

## DISCUSSION

In various studies prostaglandins have been found to stimulate gastric mucus secretion. Bolton et al. (10) measured an increase in the amount of mucus glycoproteins in the gastric lumen after treatment with prostaglandin. Using different techniques for measuring the gel thickness, prostaglandins were found to induce a rapid increase in mucus gel thickness (4,11,12). Measuring mucus secretion, however, can give technical problems. Soluble glycoproteins in gastric juice, do not necessarily reflect mucus secretion, but may also arise from mechanical or enzymatic destruction of the surface mucus gel layer. Provided that the gel structure (e.g. water content) is constant, measurement of the thickness of the gel layer may be a better method of estimating mucus secretion.

To avoid the problems inherent in measuring secretion, we have investigated the effect of prostaglandin on the biosynthesis of mucus glycoprotein. In a previous study we demonstrated that in the surface mucous cells of the rat gastric fundus [$^3$H]galactose and [$^3$H]glucosamine, but not [$^3$H]serine incorporation was increased two- to fourfold after *in vivo* 16,16-dmPGE$_2$ treatment for 1 h or 7 days (7). In the present study the incorporation of [$^3$H]galactose and [$^3$H]glucosamine was increased in the mucous cells of the isthmus of the fundus, and only slightly in the mucous neck cells.

[$^3$H]Serine incorporation was not increased in any of the cells studied. Serine is a quantitatively important amino acid of the mucus glycoprotein backbone, accounting for 13% of the amino acid residues present in this polypeptide (13). Consequently, we expected that elevated glycoprotein synthesis would lead to increased [$^3$H]serine incorporation. Analysis of purified mucus glycoproteins revealed that 16,16-dmPGE$_2$ has no effect on carbohydrate composition, oligosaccharide chain size, or buoyant density (7). In a follow-up study we showed that the synthesis of backbone polypeptides in mucus-producing cells is only a small part of total protein synthesis in these cells, which explains why an elevated synthesis of glycoprotein did not detectably increase [$^3$H]serine incorporation (14).

The observation that prostaglandins do not prevent ethanol-induced damage to the surface mucous cells (3-5), makes it doubtful whether the increased mucus glycoprotein synthesis in these cells has any significance in gastric cytoprotection against extremely harmful agents. However, prostaglandins have been reported to limit the depth of ethanol-induced mucosal injury (4), sparing the mucous cells in the isthmus and neck. Morris and Harding (15) have shown that rapid recovery of the mucosa occurs via migration of the epithelial cells from undamaged parts of the gastric foveola and from lower levels, and that a

layer of extracellular mucus is formed. We hypothesize that by stimulating the glycoprotein synthesis in the mucous cells of the isthmus, as was found in the present study, prostaglandin might accelerate the healing process in the mucosa, since the presence of a functional mucus layer could be an important factor in limiting further damage.

Ohno et al. (5) have shown by means of scanning electron microscopy that the surface mucous cells in prostaglandin-pretreated rats are protected during exposure to 30 or 100 mg of aspirin per kg body weight. To us, this suggests that elevated glycoprotein synthesis in the surface mucous cells might play a role in cytoprotection during exposure to less damaging agents.

In the present study we observed a difference between fundus and antrum after prostaglandin treatment. Whilst in the fundus 16,16-dmPGE$_2$ treatment drastically and in most cases significantly ($p < 0.05$) increased [$^3$H]galactose and [$^3$H]glucosamine incorporation in the mucous cells of the surface and isthmus, no increase or a much smaller and not statistically significant increase was seen in the antrum and duodenum. This finding is consistent with the results of McQueen et al. (12), who showed that prostaglandin induces a rapid increase in mucus gel thickness in the stomach, but does not affect the mucus gel thickness in the duodenum. Unfortunately, they did not differentiate between the fundic and antral parts of the stomach. Since in the rat lesions produced by absolute ethanol, 0.6 M HCl or aspirin are found to be located in the fundus and less commonly in the antrum (4,8), it is possible that endogenous prostaglandins are able to protect the antral mucosa, but fail to do so in the fundus. In that case exogenous prostaglandins could provide a higher level of protection in the fundus.

We conclude that a role for prostaglandin-stimulated mucus glycoprotein synthesis in protecting antral and duodenal mucosa is not very obvious. In the fundus, however, the prostaglandin-induced increase in mucus glycoprotein synthesis points to a role for mucus in cytoprotection.

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