Stimulation of glycoprotein and protein synthesis in isolated pig gastric mucosal cells by prostaglandins

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Abstract

The purpose of this study is to evaluate the effects of different prostaglandin derivatives on protein and glycoprotein synthesis and secretion in isolated and enriched pig gastric mucous cells, as measured by the incorporation of [³H]L-leucine and N-acetyl-[¹⁴C]D-glucosamine respectively into acid insoluble macromolecules (AIM). PGE₂ and 16,16-dimethyl-PGE₂ enhanced the incorporation of the amino sugar into cellular (EC₅₀ 8 and 75 nmol/l) and secreted (EC₅₀ 30 and 270 nmol/l) AIM in a concentration dependent manner during a 20 hours incubation. After incubation for eight hours or more they also stimulated the incorporation of [³H]L-leucine into cellular AIM. PGF_{2α} was considerably less potent (EC₅₀ >1 μmol/l) than the E-type prostaglandins. Iloprost, a stable prostacyclin analogue, was ineffective.

Production of mucus, especially the synthesis and secretion of gel forming, high molecular weight glycoproteins, is one of the major physiological functions of the stomach¹ and prostaglandins are known to enhance this process *in vivo*.² It remains unclear, however, whether this is caused by a direct effect of prostaglandins on the gastric mucous cells, because indirect effects, resulting from the cellular heterogeneity of the gastric mucosa and from possible interferences with other neuronal or humoral regulation systems, cannot be ruled out in *in vivo* studies. To avoid this problem, mucus production was examined directly at the cellular level,³ showing that isolated and enriched pig gastric mucous cells synthesise proteins and glycoproteins in suspension culture for up to 20 hours of incubation, as measured by the incorporation of [³H]L-leucine ([³H]Leu) and N-acetyl-[¹⁴C]D-glucosamine ([¹⁴C]GlcNAc) respectively into trichloroacetic acid/phosphotungstic acid (TCA/PTA) insoluble macromolecules (AIM). The aim of the present study was to evaluate the time and concentration dependence of prostaglandin effects on these parameters and to examine possible differences in the potency and efficacy between PGE₂, 16,16-dimethyl-PGE₂ (16,16-diM-PGE₂), PGF_{2α}, and the stable prostacyclin analogue iloprost.

Methods

CELL PREPARATION

Cells were prepared by using a slight modification of the method of Mårdh *et al.*⁴ In brief, gastric mucosal cells were isolated from pig corpus mucosa by successive treatment with

pronase and collagenase. Cell separation was achieved by counterflow centrifugation, using a Beckman elutriator rotor on a Beckman J-6 centrifuge, resulting in four cell populations (F1-F4). For this we used F2 cells, consisting of about 54% mucous cells, 42% chief cells and 4% parietal cells, as determined by cell staining,³ in a concentration of 2 × 10⁶ cells/ml. Cell viability, as checked by trypan blue exclusion, was >90%.

INCORPORATION OF RADIOACTIVE TRACERS

As previously described,³ cells were incubated in Dulbecco's modified Eagle's medium, containing HEPES 25 mmol/l instead of NaHCO₃, L-leucine 10 μmol/l, bovine serum albumin 1 mg/ml, penicillin 100 IU/ml, streptomycin 100 μg/ml and indometacin 10 μmol/l. Cell suspensions (1.8 ml) were incubated in uncapped vials in a shaking water bath at 37°C in the presence of 0.028 μCi/ml [³H]Leu and 0.278 μCi/ml [¹⁴C]GlcNAc and the test substances to be studied. Iloprost was purchased as a 1.4 mmol/l aqueous solution. The other prostaglandins were dissolved at a concentration of 10 mmol/l in ethanol 100%. All prostanoids were diluted with H₂O to the required concentrations immediately before use and added in a volume of 18 μl. Appropriate controls were run with ethanol, which had significant effects only in a concentration of 1%, corresponding to the concentration contained in the samples treated with prostaglandins 100 μmol/l. At the times indicated, cellular and secreted AIM were separated by centrifugation and precipitated by TCA/PTA (final concentration 10%/1%). The samples were left at 4°C overnight, washed thoroughly, and then solubilised in 1 ml KOH 0.6 mol/l. Incorporated radioactivity was determined in a LKB 1217 rackbeta liquid scintillation counter, using quench correction programmes for ³H and ¹⁴C labelled samples, and was expressed as dpm/10⁶ cells after correcting for unspecific trapping by the incubation medium.

STATISTICAL ANALYSIS

Analysis of variance⁵ and subsequently Dunnett's t test⁶ were used to compare the mean values of different treatments. Differences with a p < 0.05 were considered to be significant. Results were expressed as mean (SEM). EC₅₀ values were estimated graphically. Correlation coefficients were calculated by linear regression.

SUBSTANCES

Collagenase type I and V, Dulbecco's modified Eagle's medium deficient in L-leucine, penicil-

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Accepted for publication
19 June 1989

Figure 1: Time course of the incorporation of (a) [^{14}C]GlcNAc and (b) [^3H]Leu. Isolated gastric cells were incubated with the tracers for the indicated times without ($\circ$, $\bullet$) or with PGE_2 1 $\mu\text{mol/l}$ ($\square$, $\blacksquare$) or $\text{PGF}_{2\alpha}$ 1 $\mu\text{mol/l}$ ($\triangle$, $\blacktriangle$) and activity incorporated into cellular (open symbols) and suspension medium (closed symbols) AIM was determined as described under methods. Each value represents the mean of 12 separate incubations, obtained for both prostaglandins concurrently in four different cell preparations. SEM were omitted for reasons of clarity.

lin-streptomycin solution (10 000 IU/ml, 10 mg/ml), PGE_2 , $\text{PGF}_{2\alpha}$ [Sigma, München, FRG]; 16,16-diM- PGE_2 [Upjohn, Kalamazoo, Michigan, USA]; iloprost [Schering, Berlin]; bovine serum albumin, HEPES [Serva, Heidelberg, FRG]; [4,5- ^3H]L-leucine spec. act. 140 Ci/mmol in 2% ethanol, N-acetyl-[^{14}C]

D-glucosamine spec. act. 58.7 mCi/mmol in 2% ethanol [Amersham, Braunschweig, FRG]; indometacin sodium trihydrate [Merck, Sharp and Dohme, Rahway, USA]. All other substances were of analytical grade by Merck [Darmstadt, FRG].

Results

TIME COURSE OF THE EFFECTS OF PGE_2 AND $\text{PGF}_{2\alpha}$

The basal incorporation of [^{14}C]GlcNAc (Fig 1a) and [^3H]Leu (Fig 1b) into cellular AIM ($r=0.998$ and $r=0.979$) and that secreted into the suspension medium ($r=0.988$ and $r=0.992$) proceeded up to 20 hours in an almost linear fashion. PGE_2 1 $\mu\text{mol/l}$ enhanced the incorporation of [^{14}C]GlcNAc into cellular and suspension medium AIM throughout the 20 hour incubation, whereas $\text{PGF}_{2\alpha}$ 1 $\mu\text{mol/l}$ was without significant effect. A distinct stimulation of the incorporation of [^3H]Leu into cellular and suspension medium AIM under PGE_2 could be detected only after an incubation of eight hours or more. Again $\text{PGF}_{2\alpha}$ was without significant effect throughout the incubation.

CONCENTRATION DEPENDENCE OF PROSTAGLANDIN EFFECTS

PGE_2

After 20 hours of incubation PGE_2 enhanced the incorporation of [^{14}C]GlcNAc (Fig 2a) and [^3H]Leu (Fig 2b) into cellular AIM (EC_{50} 8 and 50 nmol/l) and that secreted into the suspension medium (EC_{50} 30 and 200 nmol/l) in a concentration dependent manner. PGE_2 produced maximal stimulation of [^{14}C]GlcNAc and [^3H]Leu incorporation into cellular (39%, $p<0.01$ and 42%, $p<0.01$) AIM at 1 $\mu\text{mol/l}$, whereas incorporation into AIM secreted into the suspension medium (61%, $p<0.01$ and 29%, $p<0.01$) was maximal at 10 $\mu\text{mol/l}$.

16,16-diM- PGE_2

After 20 hours of incubation, 16,16-diM- PGE_2 enhanced the incorporation of [^{14}C]GlcNAc (Fig 3a) and [^3H]Leu (Fig 3b) into cellular (EC_{50} 75 and 130 nmol/l) AIM in a concentration dependent manner, with a maximal stimulation of 45% ($p<0.01$) and 53% ($p<0.01$) respectively at 1 $\mu\text{mol/l}$. Incorporation of [^{14}C]GlcNAc into AIM secreted into the suspension medium (EC_{50} 270 nmol/l) was stimulated maximally by 32% at 10 $\mu\text{mol/l}$, however, no significant effect on the corresponding [^3H]Leu activity was detectable. Concentration response curves were bell shaped and 16,16-diM- PGE_2 100 $\mu\text{mol/l}$ significantly inhibited the [^{14}C]GlcNAc incorporation into cellular and secreted AIM and the amount of secreted acid-precipitable [^3H]Leu activity.

Although the prostaglandin enhanced the incorporation of [^{14}C]GlcNAc (Fig 4a) into cellular AIM also after four hours of incubation (EC_{50} 80 nmol/l), maximally by 37% ($p<0.01$) at 10 $\mu\text{mol/l}$, no significant stimulation of [^3H]Leu (Fig 4b) incorporation into cellular and suspen-

Figure 2: Effect of PGE_2 on the incorporation of (a) [^{14}C]GlcNAc and (b) [^3H]Leu after 20 h of incubation. Isolated gastric cells were incubated with the tracers and PGE_2 in the concentrations indicated and activity incorporated into cellular ($\circ$) and suspension medium ($\bullet$) AIM was determined as described under methods. Each value represents the mean (SEM) of eight separate incubations, obtained in three different cell preparations. * = $p<0.05$, ** = $p<0.01$ versus control.

Figure 3: Effects of 16,16-diM-PGE₂ (○, ●) and PGF_{2α} (□, ■) on the incorporation of (a) [¹⁴C]GlcNAc and (b) [³H]Leu after 20 h of incubation. Isolated gastric cells were incubated with the tracers and the prostaglandins in the concentrations indicated and activity incorporated into cellular (open symbols) and suspension medium (closed symbols) AIM was determined as described under methods (△, ▲) depicts samples treated with ethanol 1% alone. Each value represents the mean (SEM) of 12 separate incubations, obtained for both prostaglandins concurrently in 4 different cell preparations. * = *p* < 0.05, ** = *p* < 0.01 versus control.

sion medium AIM was detectable after this shorter incubation.

PGF_{2α}

After 20 hours of incubation PGF_{2α} had significant effects on the incorporation of [¹⁴C]GlcNAc (Fig 3a, 27%, *p* < 0.05) and [³H]Leu (Fig 3b, 24%, *p* < 0.05) into cellular AIM only in concentrations of 10 μmol/l and 100 μmol/l respectively and failed to enhance the incorporation of the tracers into secreted AIM.

After four hours of incubation the prostaglandin enhanced the incorporation of [¹⁴C]GlcNAc (Fig 4a) into cellular AIM again in a

Figure 4: Effects of 16,16-diM-PGE₂ (○, ●) and PGF_{2α} (□, ■) on the incorporation of (a) [¹⁴C]GlcNAc and (b) [³H]Leu after 4 h of incubation. Isolated gastric cells were incubated with the tracers and the prostaglandins in the concentrations indicated and activity incorporated into cellular (open symbols) and suspension medium (closed symbols) AIM was determined as described under methods. (△, ▲) depicts samples treated with ethanol 1% alone. Each value represents the mean (SEM) of 12 separate incubations, obtained for both prostaglandins concurrently in 4 different cell preparations. * = *p* < 0.05, ** = *p* < 0.01 versus control.

concentration of 10 μmol/l (19%, *p* < 0.05) but had no significant stimulatory effect on the incorporation of [³H]Leu (Fig 4b).

Iloprost

After 20 hours of incubation iloprost had no significant effects on the incorporation of the tracers into cellular and secreted AIM in concentrations ranging from 100 pmol/l to 10 μmol/l (Fig 5).

Discussion

Prostaglandins are synthesised ubiquitously in the body and exert a variety of biological effects.⁷ The spectrum and relative amount of different prostaglandin derivatives produced, as well as their rank order of potency⁸ however, may vary from one tissue to another. The latter seems to be the result of the existence of different prostaglandin receptors.⁹

In the stomach *in vivo* experiments indicated a stimulation of mucous glycoprotein production by PGE and PGF derivatives and this has been related to their protective effect on the gastric mucosa against various noxious agents.² E-type prostaglandins also had trophic effects as reflected in an increase in weight of the gastric mucosa,¹⁰⁻¹³ suggesting an additional influence on overall protein synthesis. Because of the cellular heterogeneity of the gastric mucosa, however, it remained unclear if these stimulations resulted from direct effects on the gastric mucous cells or were because of interactions with neuronal or humoral regulatory systems located on other cell types. To avoid these difficulties inherent to *in vivo* studies, we evaluated the potency and efficacy of PGE₂, 16,16-diM-PGE₂, PGF_{2α} and iloprost, a stable prostacyclin analogue, on protein and glycoprotein production directly at the cellular level, by using isolated and enriched pig gastric mucous cells.

GLYCOPROTEIN PRODUCTION

Besides their *in vivo* effects, E-type prostaglandins enhanced incorporation of [¹⁴C]GlcNAc and [³H]glucosamine, both amino sugar precursors of glycoproteins, *in vitro* into cellular AIM of isolated¹⁴ or cultured¹⁵ rat gastric mucosal cells. Although in these investigations stimulations were achieved only with supra-physiological concentrations of the prostanoids as high as 100 and 3 μmol/l respectively, a possible physiological function of prostaglandins in the regulation of glycoprotein synthesis was supported by an enhanced incorporation of [³H]glucosamine by cultured rat gastric cells in presence of the prostaglandin precursor arachidonic acid.¹⁶ In the present paper we could show by using enriched gastric mucous cells, that the E-type prostaglandins PGE₂ and 16,16-diM-PGE₂ enhance the incorporation of [¹⁴C]GlcNAc into cellular and secreted AIM concentration dependently already in the nanomolar range and that this stimulation is detectable throughout a 20 hour incubation. In regard of the specificity of this effect for different prostaglandin derivatives, we could show that

Figure 5: Effect of iloprost on the incorporation of (a) $[^{14}\text{C}]\text{GlcNAc}$ and (b) $[^3\text{H}]\text{Leu}$ after 20 h of incubation. Isolated gastric cells were incubated with the tracers and iloprost in the concentrations indicated and activity incorporated into cellular (○) and suspension medium (●) AIM was determined as described under methods. Each value represents the mean (SEM) of 15 separate incubations, obtained in five different cell preparations.

$\text{PGF}_{2\alpha}$ is less potent ($\text{EC}_{50} > 1 \mu\text{mol/l}$) and efficacious and that the stable prostacyclin analogue iloprost is ineffective in concentrations up to $10 \mu\text{mol/l}$. This is in accordance with a study in which $16,16\text{-diM-PGE}_2$ was also more potent than $\text{PGF}_{2\alpha}$ in stimulating the secretion of prelabelled glycoproteins from gastric mucosal explants of the rabbit gastric mucosa, although both prostaglandins failed to enhance glycoprotein synthesis in this investigation.¹⁷

PROTEIN PRODUCTION

Data on the *in vivo* effect of prostaglandins on overall protein synthesis are scarce. An autoradiographic investigation in the rat indicated that $16,16\text{-diM-PGE}_2$ had no effect on incorporation of $[^3\text{H}]\text{serine}$ into proteins after one hour of treatment, but that it enhanced the incorporation of the labelled amino acid by surface mucous cells after seven days of treatment by 80%, although the authors refused to accept this increase as significant.¹⁸ In *in vitro* experiments, an enhanced incorporation of labelled amino acids under $16,16\text{-diM-PGE}_2$ or PGE_2 was found by Tsukada *et al.*,¹⁹ using a crude cell preparation of the rat stomach, and by Bersimbaev *et al.*¹⁴ working with isolated rat gastric mucosal cells, although in the latter study stimulation required the high concentration of $100 \mu\text{mol/l}$ of the prostanoid. In our investigation the E-type prostaglandins enhanced cellular protein synthesis already in the nanomolar range. Although a distinct stimulation of the incorporation of $[^3\text{H}]\text{Leu}$ into cellular AIM by PGE_2 and $16,16\text{-diM-PGE}_2$ was only detectable after eight hours or more of incubation and EC_{50} values seemed to be higher than that for stimulation of glycoprotein synthesis, the specificity of this effect was the same as that for $[^{14}\text{C}]\text{GlcNAc}$ incorporation, as iloprost was again ineffective and $\text{PGF}_{2\alpha}$ was less potent and efficacious than the E-type

prostaglandins. These *in vitro* results are in accordance with an *in vivo* investigation, in which prostacyclin and the stable prostacyclin derivative Hoe 982 failed to enhance the growth of rat gastric mucosa, whereas PGE and PGF derivatives were effective.¹³

Our results suggest that stimulation of glycoprotein and protein synthesis and secretion are PGE receptor mediated processes. This view is supported by the demonstration of specific PGE binding sites in homogenates of pig²⁰⁻²² and on isolated cells of canine²³ and rat¹⁴ gastric mucosa. No specific binding sites for F-type prostaglandins could be detected²⁴ and binding sites for I-type prostaglandins²⁵ are at least controversial.²⁶ Our results offer no clues how the prostaglandins stimulate glycoprotein and protein synthesis. The underlying mechanism(s) as well as the nature of the synthesised radiolabelled products remain to be evaluated. Stimulation does not seem to be inevitably related to an increase in DNA synthesis, however,^{11 27-29} cell turnover,³⁰ or cell number¹² in the gastric mucosa. Cyclic AMP may be a possible intracellular 'second messenger' for the prostaglandins, although the physiological importance of the reported stimulations^{14 15 31} remains to be established.

From the stimulatory actions of PGE_2 and $16,16\text{-diM-PGE}_2$ in the nanomolar range and the well known fact that gastric mucosal cells synthesize prostaglandins, mainly PGE_2 and PGI_2 ,³² we conclude that E-type prostaglandins may be involved in the physiological regulation of mucus production in the stomach *in vivo*, whereas prostacyclin may have other targets for action – for example, gastric microcirculation.³²

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