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# Prostaglandin formation by isolated gastric parietal and nonparietal cells of the rat

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**1** Rat gastric cells isolated by pronase and subdivided by Percoll into 3 fractions (F1, F2, F3) were used to study prostaglandin $E_2$ ($PGE_2$) formation and, as an indirect measure of parietal cell $H^+$ production, [$^{14}$C]-aminopyrine uptake.

**2** Cells that had not been fractionated, with 20 to 25% parietal cells, contained at 0°C $1.7 \pm 0.35$ (s.e.mean) ng $PGE_2$ $10^8$ cells$^{-1}$. During incubation at 37°C these cells steadily synthesized up to $4.36 \pm 0.73$ ng $PGE_2$ $10^8$ cells$^{-1}$ from endogenous substrate.

**3** Indomethacin in concentrations higher than $10^{-6}$ mol $l^{-1}$ inhibited this basal formation completely, but $10^{-4}$ mol $l^{-1}$ did not reduce the cellular $PGE_2$ level below $1.4 \pm 0.2$ ng $10^8$ cells$^{-1}$.

**4** Arachidonic acid in concentrations higher than $10^{-5}$ mol $l^{-1}$ evoked an abundant formation of $PGE_2$, and $10^{-4}$ mol $l^{-1}$ built up a plateau of over $7.5 \pm 1.65$ ng $PGE_2$ $10^8$ cells$^{-1}$ within 15 min.

**5** $PGE_2$ formation in cell fractions increased significantly with the number of parietal cells per assay tube.

**6** Indomethacin ($10^{-8}$ to $10^{-4}$ mol $l^{-1}$) did not influence the histamine-stimulated uptake of [$^{14}$C]-aminopyrine, while arachidonic acid ($10^{-5}$ to $10^{-4}$ mol $l^{-1}$) inhibited this process.

**7** $PGE_2$ formation in response to arachidonic acid was prevented by indomethacin, but the inhibition of aminopyrine uptake by arachidonic acid could not be prevented by indomethacin.

**8** The data suggest that isolated gastric cells of the rat sustain constant $PGE_2$ synthesis *in vitro*, which is more pronounced in parietal than in mucosal and chief cells. $PGE_2$ may exert different effects within distinct gastric cell types.

## Introduction

Prostaglandins of the E series are synthesized by the gastric mucosa and released into the gastric juice of man and other mammalian species (Bennett *et al.*, 1973; Baker *et al.*, 1977). Natural prostaglandins have been shown to inhibit basal and stimulated gastric acid and pepsin secretion (Robert, 1974; 1975; Konturek *et al.*, 1976), an effect requiring rather high prostaglandin concentrations. At much lower concentrations, prostaglandins have a cytoprotective action on the gastric mucosa against various damaging factors (Robert, 1975). Thus, prostaglandins may have at least a dual function within the gastric mucosa: (1) a regulatory and/or modulatory role in the control of acid secretion and (2) a cytoprotective role in the maintenance of gastric mucosal integrity.

A number of investigations have demonstrated gastric mucosal production of prostaglandin (Pace-Asciak & Wolfe, 1971; Peskar, 1977) including production by canine isolated gastric cells (Skoglund *et al.*, 1980). Our recent findings have also indicated mucosal prostaglandin synthesis, since the intragastric instillation of aluminium hydroxide caused an enhanced prostaglandin $E_2$ ($PGE_2$) level in the mucosa and gastric juice with a concomitant antiulcer effect, pointing to an additional unknown mechanism of this antacid (Szelenyi *et al.*, 1983). However, in these investigations there was no possibility of evaluating the type of cell responsible for prostaglandin formation. Therefore, the aim of the present work was mainly to study prostaglandin synthesis of rat isolated gastric cells under different conditions in order to establish an *in vitro* system for $PGE_2$ formation and to identify its cellular origin.

## Methods

### *Cell isolation*

Three different cell media were used. Medium A (mmol $l^{-1}$): $NaH_2PO_4$ 0.5, $Na_2HPO_4$ 1.0, $NaHCO_3$ 20, NaCl 70, KCl 5, glucose 11, EDTA 2, Hepes 50, and bovine serum albumin (20 mg $ml^{-1}$). Medium B was of the same composition as medium A, but was EDTA-free and contained additionally $CaCl_2$ 1.0 and $MgCl_2$ 1.5. Medium C differed from medium B in having a reduced albumin concentration (1 mg $ml^{-1}$).

Cell isolation was performed according to the method of Lewin *et al.* (1974). Briefly, 10 nonfasted female Wistar rats (FW 49 Biberach, 230 to 280g) were used per experiment and killed by decapitation. The stomachs were removed, transformed into everted sacs, filled with pronase solution (11 mg $ml^{-1}$) and incubated at 37°C in medium A gassed with 95% $O_2$ and 5% $CO_2$. The incubation lasted 90 min and medium A was renewed twice. The sacs were then transferred to medium B and gently stirred for 30 min by a magnetic stirrer. During this step the cells were isolated and collected by centrifugation. The number of cells and the percentage of parietal cells and dead cells (trypan blue) were estimated by cell count. The parietal cells (20–25%) were readily identified under the light microscope by their unique morphological features. The isolated gastric cells (FO, unfractionated) were stored at 0°C in medium C, which was used as standard buffer for all experiments.

### *Cell fractionation*

To obtain parietal cell-poor and enriched cell fractions, isopycnic centrifugation with Percoll was carried out as described by Sonnenberg *et al.* (1979). Cell-containing medium C (6.5 ml) was mixed with 2.7 ml Percoll and 0.8 ml Hepes (0.25 mol $l^{-1}$; density 1.03 g $ml^{-1}$), and was followed by centrifugation at 200 *g* for 20 min. This resulted in fractions with a different content of parietal cells: the top of the separation mixture over 80% (F3), within the supernatant portion (F2) less than 25%, and at the bottom less than 10% (F1). In all fractions in these experiments only the parietal cells were quantified. However, we know from other investigations and cell staining that F1 contains the bulk of chief cells, whereas F2 consists predominantly of mucous cells (Schepp *et al.*, 1983a). The fractions were carefully washed free from Percoll before use. Not more than 5% of these cells were damaged.

### *Determination of prostaglandin $E_2$*

Cell incubations ($10^7$ cells 1.1 $ml^{-1}$ medium C) were stopped by freezing the test tubes of thin glass and their contents in liquid nitrogen. The samples were then lyophilized and stored at − 20°C until being assayed for $PGE_2$. To prepare the samples, they were homogenized in the presence of 75% ethanol (Green *et al.*, 1978) and glass beads at − 196°C in a Teflon capsule by means of a microdismembrator (Braun, Melsungen) for 30 s. After controlled thawing up to − 10°C, the content was diluted to 25% ethanol and a second homogenization and extraction step performed for 15 s. After centrifugation at 5000 *g* for 10 min at 0°C, 10 to 100 µl aliquot portions of the supernatant fluids were assayed in duplicate by radioimmunoassay (Dray *et al.*, 1975). Standards in the range of 0.24 to 125 pg $PGE_2$ were treated in the same way as the samples, and the curve was calculated by an iterative logit-log standard programme of a Kontron MR 480 gamma counter. The limit of detection of $PGE_2$ was 0.2 pg per assay tube, nonspecific binding was 0.86 ± 0.40%, $PGE_2$ recovery was over 95%, cross reactivity with $PGE_1$ was 10.7% and with all other prostaglandins less than 0.3%; the intra-assay variation was 1.60 ± 0.84% (s.e.mean) and the inter-assay variation was 1.64 ± 1.1%.

### [$^{14}$C]-*aminopyrine (AP)-uptake*

The method of Berglindh *et al.* (1976) was used in modified form: 400 µl separated cells ($10^7$ $ml^{-1}$) were incubated in medium C at 37°C in the presence of 0.04 µCi [$^{14}$C]-AP. After 20 min preincubation the test compounds were added and the reaction continued up to 40 min. AP-uptake was stopped by cell centrifugation (5000 *g*), the supernatant fluid was discarded and the cells were washed once by resuspension in medium C. The washed sediment was then treated with 400 µl Lumasolve and, after 12 h, radioactivity was monitored in a liquid scintillation counter (Mark II, Nuclear Chicago). [$^{14}$C]-AP-uptake is expressed in cpm $10^{-6}$ cells.

### *Drugs*

$PGE_2$ was purchased from Sigma (Taufkirchen, FRG), [5, 6, 8, 11, 12, 14, 15, (n)-$^3$H]-prostaglandin $E_2$, sp. act. 6.2 TBp $mmol^{-1}$ and [dimethylamine $^{14}$C]-aminopyrine, sp. act. 4.1 GBp $mmol^{-1}$ from Amersham Buchler (Braunschweig, FRG), and the $PGE_2$ antiserum from the Institute Pasteur Production by Fresenius (Bad Homburg, FRG). Rialuma scintillation mixture was from Baker Chemicals (Deventer, The Netherlands), pronase E from Merck (Darmstadt, FRG) and Percoll from Pharmacia Fine Chemicals (Uppsala, Sweden). All other substances were Analar grade chemicals from Merck (Darmstadt, FRG), Serva (Heidelberg, FRG) or Sigma (Taufkirchen, FRG).

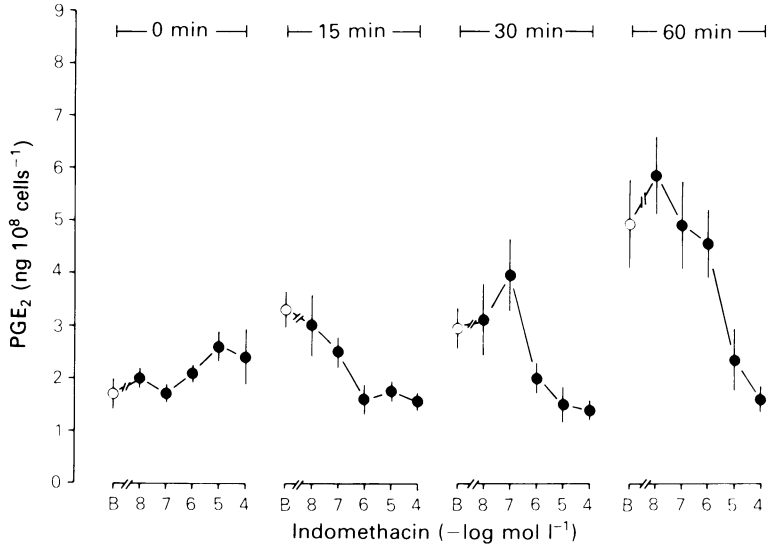


**Figure 1** Influence of indomethacin ($10^{-8}$ to $10^{-4}$ mol $l^{-1}$) on basal prostaglandin $E_2$ ($PGE_2$) formation (B) of isolated gastric mucosal cells of rat. The cells were incubated at 37°C for different periods of time; at 0 min they were kept in ice before inactivation in liquid nitrogen. Each point represents the mean of $n = 6$ incubations of a cell mixture prepared from 20 rat stomachs; s.e.means shown by vertical lines. Parietal cell content was 22%.

*Statistical analysis*

The results are expressed as means ± s.e.mean. Statistical significance of the effects of any intervention was determined by using Student's *t* test for unpaired data.

## Results

*Prostaglandin $E_2$ formation in non-fractionated cells*

*Basal $PGE_2$ formation* The gastric cell mixture steadily produced $PGE_2$ and during 60 min of incubation the level rose from below 2 ng $10^8$ $cells^{-1}$ up to 4.9 ± 0.65 (Figure 1) or 3.82 ± 0.81 (Figure 2), which is an average of 156 ± 42% ($P < 0.01$; Figure 3).

*Effect of indomethacin* Indomethacin inhibited $PGE_2$ synthesis and inhibition was already apparent during the first 15 min of incubation. Figure 1 clearly shows that it reduced the cellular $PGE_2$ formation in a concentration-dependent manner. The threshold concentration was about $10^{-7}$ mol $l^{-1}$, whereas $10^{-4}$ mol $l^{-1}$ inhibited $PGE_2$ synthesis maximally. The approximate $IC_{50}$ for indomethacin was $3 \times 10^{-6}$ mol $l^{-1}$ as calculated from the 60 min experiments. Indomethacin in a concentration of $10^{-5}$ mol $l^{-1}$ was as effective as $10^{-4}$ mol $l^{-1}$ after 15 and 30 min of incubation, but not after 60 min (Figure 3).

*Effect of arachidonic acid (AA)* Figures 2 and 3 show the effect of exogenous AA on $PGE_2$ synthesis by gastric mucosal cells. AA caused a concentration- and time-dependent increase, although concentrations higher than $10^{-5}$ mol $l^{-1}$ were necessary. $PGE_2$ synthesis was maximally increased within 15 min by $10^{-4}$ mol $l^{-1}$ AA. Longer incubation periods did not further enhance $PGE_2$ synthesis. After 60 min of incubation, the mean basal $PGE_2$ level of 3.82 ± 0.87 ng $10^{-8}$ $cells^{-1}$ was significantly ($P < 0.05$) below the $PGE_2$ concentration of 8.77 ± 2.5 ng $10^{-8}$ $cells^{-1}$ produced by $10^{-4}$ mol $l^{-1}$ AA. If the data were expressed as a percentage of the starting level as shown in Figure 3, the differences were less pronounced and a significant stimulation in the presence of $10^{-4}$ mol $l^{-1}$ AA was found only after 15 min of incubation ($P < 0.05$).

*Effect of arachidonic acid plus indomethacin* Non-fractionated cells prepared from 10 rat stomachs and containing 23.4% parietal cells were incubated for 60 min and the influence of indomethacin on the $PGE_2$ synthesis in response to AA was investigated. The following data were obtained (ng $PGE_2$ $10^8$ $cells^{-1}$ $h^{-1}$, mean ± s.e.mean, $n = 6$): basal formation, 6.06 ± 2.71; with indomethacin $10^{-5}$ mol $l^{-1}$, 3.31 ± 1.74; with AA, $10^{-4}$ mol $l^{-1}$, 11.81 ± 2.56; with AA, $10^{-4}$ mol $l^{-1}$ and indomethacin, $10^{-5}$ mol $l^{-1}$, 6.51 ± 1.02, indicating significant inhibition by indomethacin ($P < 0.05$).

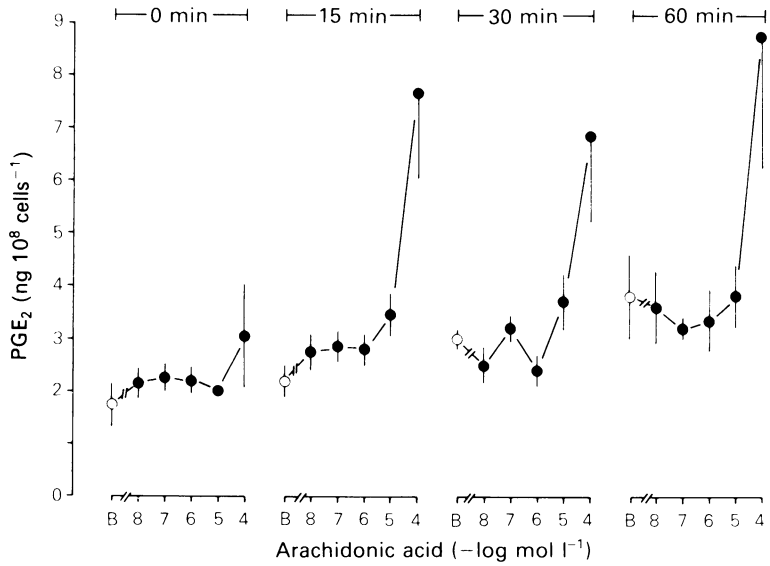


**Figure 2** Influence of arachidonic acid ($10^{-8}$ to $10^{-4}$ mol $l^{-1}$) on basal prostaglandin $E_2$ ($PGE_2$) formation (B) of isolated gastric mucosal cells of rat. The cells were incubated at 37°C for different periods of time; at 0 min they were kept in ice before inactivation in liquid nitrogen. Each point represents the mean of $n = 6$ incubations of a cell mixture prepared from 20 rat stomachs; s.e.means shown by vertical lines. Parietal cell content was 24%.

*Prostaglandin $E_2$ formation in fractionated cells with different content of parietal cells*

Basal $PGE_2$ formation, and the effects of indomethacin ($10^{-5}$ mol $l^{-1}$) and AA ($10^{-5}$ mol $l^{-1}$) on $PGE_2$ formation in fractions with different parietal cell compositions incubated for 60 min, are shown in Figure 4. Under all conditions the highest $PGE_2$ formation was found in the parietal cell-rich fraction F3, whereas the parietal cell-poor fractions F1 and F2, which contain mostly chief and mucous cells, produced considerably less $PGE_2$. $PGE_2$ formation (ng $10^8$ cells$^{-1}$ $h^{-1}$) without (basal) and in the presence of $10^{-5}$ mol $l^{-1}$ indomethacin correlated with the number of parietal cells per assay tube ($P < 0.05$). The effect of indomethacin ($10^{-5}$ mol $l^{-1}$) was not very pronounced in these fractionated cells, i.e. basal $PGE_2$ formation was not reduced in all fractions tested. Under the influence of $10^{-5}$ mol $l^{-1}$ AA, all fractions responded with enhanced $PGE_2$ synthesis, and again the enriched parietal cells were the most effective. However, within these experiments correlation of the amounts of $PGE_2$ synthesized with the parietal cell number did not quite reach the level of statistical significance.

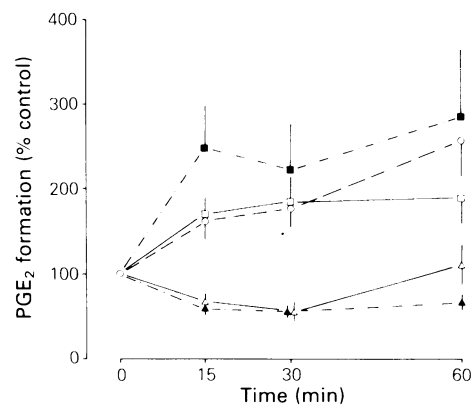


**Figure 3** Prostaglandin $E_2$ ($PGE_2$) formation under basal conditions (○) and the influence of $10^{-5}$ mol $l^{-1}$ (△) and $10^{-4}$ mol $l^{-1}$ (▲) indomethacin or $10^{-5}$ mol $l^{-1}$ (□) and $10^{-4}$ mol $l^{-1}$ (■) arachidonic acid in isolated gastric cells of rat expressed as a percentage of the corresponding value at 0 min (100%). The figure combines data from Figures 1 and 2. See legends of these figures for further details.

*[$^{14}$C]-aminopyrine uptake by isolated cells*

*Effect of indomethacin* Using non-fractionated cells with 19 to 24% parietal cells, indomethacin (Figure 5a) in concentrations of $10^{-8}$ to $10^{-4}$ mol $l^{-1}$ did not change significantly the basal and histamine ($10^{-4}$ mol $l^{-1}$)-stimulated AP uptake, although there was a tendency to an increased histamine effect induced by indomethacin in the range of $10^{-6}$ to $10^{-5}$ mol $l^{-1}$.

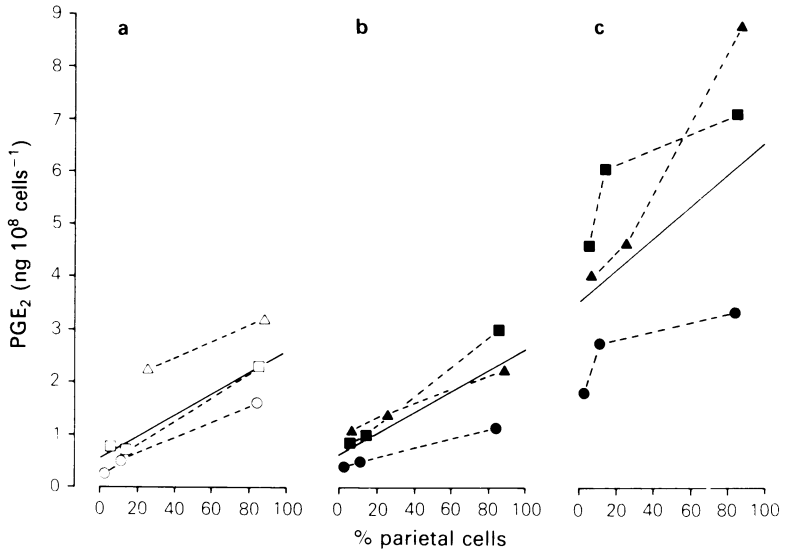


**Figure 4** Prostaglandin $E_2$ ($PGE_2$) formation under basal conditions (a) and the influence of $10^{-5}$ mol $l^{-1}$ indomethacin (b) or archidonic acid (c) in isolated gastric cells of rat with increasing content of parietal cells incubated for 60 min. In (a) (basal formation), $y = 0.02x + 0.56$, $r = 0.81$, $P < 0.05$; in (b) (indomethacin $10^{-5}$ mol $l^{-1}$) $y = 0.02x + 0.66$, $r = 0.79$, $P < 0.05$; in (c) (arachidonic acid $10^{-5}$ mol $l^{-1}$) $y = 0.03x + 3.54$, $r = 0.60$, $P < 0.05$.

Three different cell preparations, each a cell mixture from 10 rat stomachs which were separated by Percoll into 3 fractions (F1, F2, F3). Parietal cell content (mean ± s.e.mean): F1 = 5.2 ± 1.3%, F2 = 16.7 ± 4.3%, F3 = 85.6 ± 1.0%. Each symbol represents the mean of 2 incubations.

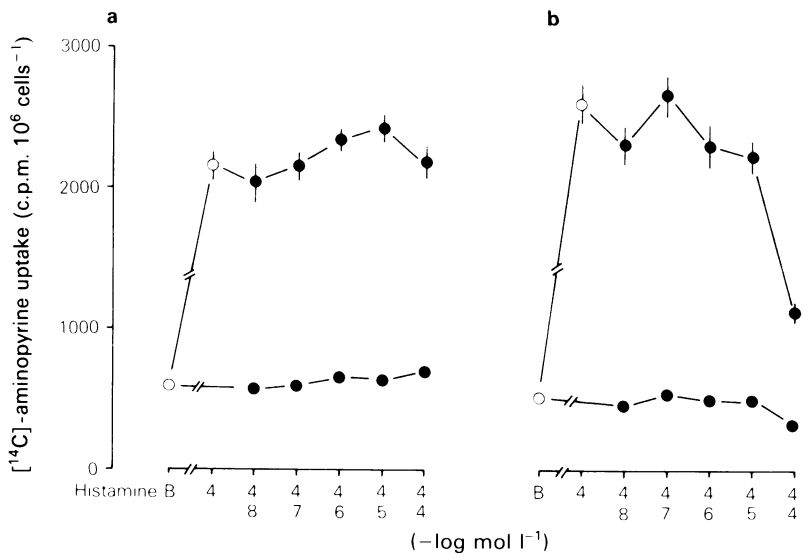


**Figure 5** Influence of $10^{-8}$ to $10^{-4}$ mol $l^{-1}$ indomethacin (a) or arachidonic acid (b) on basal (B) and histamine ($10^{-4}$ mol $l^{-1}$)-stimulated [$^{14}$C]-aminopyrine uptake. After 20 min preincubation, the drugs were added and the reaction stopped after a further period of 40 min. Each point represents the mean of $n = 6$ incubations of a cell mixture prepared from 10 rat stomachs; s.e.means shown by vertical lines. Parietal cell content was 24% (indomethacin) and 20% (arachidonic acid).

*Effect of arachidonic acid* AA, in a concentration of $10^{-4}$ mol $l^{-1}$, reduced the basal AP uptake by 38% and AA, in concentrations of $10^{-5}$ and $10^{-4}$ mol $l^{-1}$, reduced the histamine-stimulated AP uptake by 22 and 55% (Figure 5b). For all values, $P$ was $<0.01$.

*Effect of arachidonic acid plus indomethacin.* Indomethacin ($10^{-6}$ to $10^{-4}$ mol $l^{-1}$) was unable to prevent the antagonistic activity of AA ($10^{-5}$ and $10^{-4}$ mol $l^{-1}$) on the histamine ($10^{-5}$ and $10^{-4}$ mol $l^{-1}$)-stimulated [$^{14}$C]-AP uptake in time course studies up to 60 min. Also pre-incubation of the cells with indomethacin had no inhibitory effect.

## Discussion

The results show that mixtures of all different gastric mucosal cell types steadily synthesize $PGE_2$ from endogenous substrate during incubation. This process of $PGE_2$ generation is suppressed by indomethacin and stimulated by AA. The studies with fractionated cells suggest that both cell types, parietal and non-parietal cells, are able to synthesize $PGE_2$ but that there are differences in the quantity of $PGE_2$ formed by the different types of cell. Fractions with non-parietal cells, consisting mainly of chief and mucous cells, produced approximately half that of fractions containing over 80% parietal cells. These findings are largely in agreement with the results of Skoglund *et al.* (1980), who studied canine isolated gastric cells. However, Skoglund *et al.* (1980) found a 15 fold higher $PGE_2$ level in their canine isolated gastric mucosal cells. Differences in experimental design and conditions used may be the cause of this variation.

Prostaglandins have been postulated to have an important role in gastric physiology (see Miller, 1983). According to our results, $PGE_2$ can be synthesized by parietal and nonparietal cells, and both types are able to use endogenous or exogenous AA. In both cell types, indomethacin is an inhibitor of the $PGE_2$ synthesis which could offer an explanation for its ulcerogenic effect. However, in this respect, it is not easy to understand, why indomecathin depressed the level of $PGE_2$ maximally only by 42% below the initial cell concentration.

According to our results the parietal cell-enriched fractions produced more $PGE_2$ per cell than the nonparietal cell fractions. Thus, it is tempting to see this higher $PGE_2$ forming capacity in connection with the main function of parietal cells, i.e. $H^+$ production.

$PGE_2$ inhibits acid secretion *in vivo* (Main & Whittle, 1973) and also *in vitro* as measured by [$^{14}$C]-AP uptake in isolated gastric cells of dog (Soll, 1980; Skoglund *et al.*, 1982), rabbit (Levine *et al.*, 1982) and rat (Schepp *et al.*, 1983b). In rat cells, $PGE_2$ caused a concentration-dependent inhibition of the histamine ($10^{-4}$ mol $l^{-1}$)-stimulated AP uptake. Reduction was initiated by $10^{-10}$ mol $l^{-1}$ and the $IC_{50}$ value was $7 \times 10^{-8}$ mol $l^{-1}$ (Schepp *et al.*, 1983b). Such concentrations are not only likely to occur in the gastric mucosa (Peskar, 1981) but also in our present *in vitro* system with a calculated $PGE_2$ concentration of approximately $3 \times 10^{-8}$ mol $l^{-1}$ per total incubation volume in the presence of $10^{-4}$ mol $l^{-1}$ AA.

According to our experimental design it is not possible to state anything about the degree of $PGE_2$ released into the medium. Levine *et al.* (1982) who used isolated rabbit fundic glands found that only 15% of the total $PGE_2$ was released into the medium within 60 min, which would indicate that the cellular $PGE_2$ concentration in our experiments was rather higher than the calculated value per total incubation volume.

It has been shown previously in different species including man, that prostaglandins inhibit the histamine $H_2$-receptor coupled adenylate cyclase in nanomolar concentrations (Major & Scholes, 1978; Soll, 1981; Becker & Ruoff, 1982). Thus, our results suggest that parietal cells produce their own $PGE_2$, which in turn may modulate the activity of the histamine-sensitive adenylate cyclase in the same cells, thereby regulating gastric acid secretion.

This assumption is only partly supported by the present studies on [$^{14}$C]-AP uptake. The same concentrations of AA ($10^{-5}$ to $10^{-4}$ mol $l^{-1}$) which stimulated $PGE_2$ formation, also caused a reduction of the histamine-stimulated $H^+$ production. However, basal $PGE_2$ formation seems not to reach sufficient concentrations to inhibit the histamine respose, since indomethacin did not enhance the AP uptake. Also indomethacin did not prevent the inhibitory activity of AA, and therefore it appears that the effect of AA on AP uptake is not mediated by $PGE_2$ or any prostaglandin.

In another study of Skoglund *et al.* (1982) with canine gastric cells, AA initiated significant inhibition at concentrations of $10^{-6}$ mol $l^{-1}$ which may be explained by the lower concentration of histamine ($10^{-5}$ mol $l^{-1}$) used to stimulate AP uptake. In their experiments, $10^{-5}$ mol $l^{-1}$ indomethacin prevented the AA inhibition. Why our rat cells did not respond in such a way is not clear. However, it could be that in the presence of AA, parietal cell $PGE_2$ synthesis of the rat is not sufficiently depressed by $10^{-5}$ to $10^{-4}$ mol $l^{-1}$ indomethacin (Chandrabrose *et al.*, 1980) or that both compounds together in such high concentrations damage intracellular structures necessary for $H^+$ production. However, cell defects could not be detected by light microscopy (trypan blue uptake). Parietal cells represent about 20% of the heterogeneous mucosal cell population. The rest consists of different cell types, which are smaller in size and could not be quantified in the present study. Most of them are

epithelia and mucous cells, and the prostaglandins synthesized locally by these cells may have a cytoprotective function.

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