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Effect of omeprazole on eicosanoid formation in and release from guinea-pig gastric mucosal cells

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1 Guinea-pig gastric mucosal cells isolated by collagenase and pronase digestion were used to study the release of prostanoids prostaglandin I_2 (PGI_2; measured as 6-keto $PGF_{1\alpha}$), PGE_2, $PGF_{2\alpha}$ and thromboxane A_2 (TXA_2; measured as TXB_2). Lysophosphatide acyltransferase (LAT) and phospholipase A_2 (PLA_2) were measured in the microsomal fraction of isolated but not separated gastric cells and isolated and enriched parietal and mucous cells.

2 In all cell preparations PLA_2 activity was approximately 5 times higher than that of LAT.

3 Acid-activated omeprazole inhibited LAT in a concentration-dependent manner with similar IC_{50} values in gastric, parietal and mucous cells. It had no effect on PLA_2.

4 Gastric cells constantly produced PGI_2, PGE_2, $PGF_{2\alpha}$ and TXA_2. The main prostaglandins released were PGI_2 and PGE_2. $PGF_{2\alpha}$ and TXA_2 were released in smaller quantities.

5 Omeprazole dissolved in polyethylene glycol 400 (PEG) pH 2 inhibited spontaneous PGI_2 release in a concentration-dependent manner with an IC_{50} of $14.3 \pm 4.8\ \mu M$. Only concentrations as high as $100\ \mu M$ produced a significant reduction in PGE_2 release by 60%. No significant changes could be detected in the spontaneous release of $PGF_{2\alpha}$ and TXA_2.

6 Omeprazole dissolved in PEG pH 7 had no effect on PGI_2 release except at $100\ \mu M$ which led to an insignificant decrease by 40%.

7 These data suggest that omeprazole beyond its inhibitory effect on parietal cell K^+/H^+-ATPase also affects gastric mucosal prostanoid formation and release. The inhibitory effect on PGI_2 does not support the view that omeprazole protects the gastric mucosa by increasing prostanoid formation.

Introduction

Omeprazole is a potent inhibitor of gastric acid secretion in various animal models (Larsson *et al.*, 1982) and in man (Lind *et al.*, 1983). This compound, like other substituted benzimidazoles, inhibits acid secretion by blocking the K^+/H^+-ATPase in parietal cells (Fellenius *et al.*, 1981; Beil & Sewing, 1984). Before these compounds can block the K^+/H^+-ATPase they need to be converted into the active principle, which is formed in the parietal cell tubulovesicles at a low pH (Wallmark *et al.*, 1984; Rackur *et al.*, 1985; Figala *et al.*, 1986). Mercaptanes, such as β-mercaptoethanol and dithiothreitol, were found to prevent and to reverse inhibition of parietal cell acid formation and K^+/H^+-ATPase blockade, indicating that sulphydryl groups are most probably involved in the chemical reactions leading to inhibition of acid secretion (Wallmark *et al.*, 1984; Im *et al.*, 1985). Furthermore benzimidazole derivatives were shown to act as mild irritants to protect the gastric mucosa against damage caused by various necrotizing agents (Ruwart *et al.*, 1982; Konturek *et al.*, 1983, Mattsson *et al.*, 1983). The mechanism is unknown.

The purpose of this study was to examine if this effect could be associated with an increased release of prostaglandins and if so whether such an effect can be demonstrated for omeprazole itself (dissolved at pH 7) or whether – as necessary for K^+/H^+-ATPase inhibition – omeprazole requires acid activation (dissolved at pH 2) to influence eicosanoid formation and release. The mechanism responsible for an increased release of prostaglandins could be as follows. In an early step in the arachidonic acid cascade phospholipase A_2 (PLA_2) cleaves fatty acids including arachidonic acid from phospholipids i.e. phosphatidylcholine (lecithin). Arachidonic acid can be utilized for prostaglandin synthesis or reincorporated into the lyso-compounds for resynthesis of phospholipids. The latter effect is brought about by lysophosphatide acyltransferase

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(LAT). In contrast to PLA_2, LAT is an enzyme reacting with SH-reagents. In other systems such as rat peritoneal macrophages (Körner *et al.*, 1984) and W1–38 human lung fibroblasts (Hunter *et al.*, 1984) it has been shown that inhibition of LAT resulted in an increased release of prostaglandins which can be explained by an increased availability of arachidonic acid.

Methods

Cell separation

The experiments were performed with male guinea-pigs (210–310 g). Isolated and enriched parietal and mucous cells from the guinea-pig were prepared by a method similar to that of Soll (1978) with modifications described in detail by Sewing *et al.* (1983). Briefly, guinea-pig mucosal cells were isolated by collagenase and pronase digestion and separated by zonal centrifugation with the Beckman elutriator system. The procedure resulted in a parietal cell-rich fraction of 76% parietal cells, and among others a non-parietal cell fraction consisting of 96% mucous cells. Viability judged by trypan blue exclusion was greater than 95%.

Preparation of the microsomal fraction

The different cells harvested from the elutriator were placed in a medium consisting of 0.25 M sucrose containing 20 mM Tris-HCl buffer (pH 7.4). Homogenization was performed by sonification (3 × 10 s, 30 W). Subcellular fractions from a cell homogenate were obtained by differential centrifugation as follows: two centrifugations at 20,000 *g* for 20 min (pellet: cell debris, nuclear and mitochondrial fraction), the collected supernatants were recombined and centrifuged at 100,000 *g* for 60 min. The remaining pellet consisting of microsomes, was taken up in the sucrose/Tris-HCl buffer and used for enzyme studies.

Acyl-Co A:1-acyl-sn-glycero-3-phosphorylcholine acyltransferase (EC 2.3.1.23) assay

The activity of acyltransferase was determined by reacting arachidonoyl-Co A with labelled L-lyso-3-phosphatidylcholine-1-[1-^{14}C] palmitoyl. The incubation mixture contained in a total volume of 0.5 ml the following components: 0.1 mM L-a-lysophosphatidylcholine, 0.82 μM L-lyso-3-phosphatidylcholine, 1-[1-^{14}C] palmitoyl, 20 mM Na-acetate buffer pH 6, 140 mM KCl, 25 μM arachidonoyl-Co A and 10 μg enzyme protein. The reaction was started with arachidonoyl-Co A. After 10 min incubation at 37°C the reaction was stopped by the addition of 2.5 ml methanol.

Phospholipase A_2 (phosphatide 2-acylhydrolase, EC 3.1.1.4) assay

The activity of phospholipase A_2 was determined by the release of [^{14}C]-oleic acid from L-3-phosphatidylcholine, 1-palmitoyl-2-[1-^{14}C]-oleoyl. The incubation mixture contained in a total volume of 0.5 ml the following components: 0.53 mM L-a-phosphatidylcholine, 0.88 μM L-3-phosphatidylcholine, 1-palmitoyl-2-[1-^{14}C]-oleoyl, 0.35% Na-cholate, 1 mM $CaCl_2$, 50 mM Tris/HCl buffer pH 8 and 10 μg enzyme protein. The reaction was started by the addition of phospholipids and Na-cholate. After 10 min incubation at 37°C the reaction was stopped by addition of 2.5 ml methanol.

Omeprazole was dissolved at pH 7 (to leave the original structure intact) or pH 2 (to convert omeprazole, as there is a low pH in parietal cell tubulovesicles, into the active principle = acid activation) for inhibition studies on LAT in dimethylsulphoxide (DMSO) and on PLA_2 in polyethylene glycol (PEG). In either case the solution was allowed to stand for 30 min at room temperature before use. Subsequently omeprazole was preincubated with the enzyme protein for 45 min.

LAT was assayed at its optimum pH, pH 6, and PLA_2 at pH 8. However, in one set of experiments LAT was assayed at the physiological pH of 7.4.

Enzyme activities were expressed as nmol mg^{-1} protein min^{-1}.

Lipid extraction and chromatography

Phospholipids from the individual cell preparations were extracted according to Goppelt & Resch, (1984). In brief, 2.5 ml methanol was added to the incubation mixture at the end of a 10 min incubation period and vigorously shaken. Then 3.5 ml chloroform and 50 μl of a lipid mixture containing oleic acid 35.5 μM, L-a-phosphatidylcholine about 7.9 μM, and L-a-lysophosphatidylcholine about 12 μM dissolved in chloroform: methanol 2:1 were added and vigorously shaken. After 5 min 1.25 ml 0.1 M KCl was added for washing and again the mixture was shaken. Phase separation was achieved by cooling the tubes in a refrigerator for 5 min and subsequently heating them to 60°C in a water bath. The methanol-water phase (top) was discarded; the methanol-chloroform phase (bottom) was evaporated under a stream of nitrogen.

Separation of individual phospholipid species by one-dimensional thin-layer chromatography

The lipids were redissolved in 3 × 200 μl chloroform: methanol 2:1, the solution was concentrated in an Eppendorf tube under a stream of nitrogen and refilled to a volume of 50 μl with chloroform:methanol 2:1.

The total volume was applied by a CAMAG Linomat III automatic applicator to thin layer plates (silica gel t.l.c. plates F 1500, Schleicher & Schuell) which were dried and cooled to 4°C in glass tanks. The filter-paper-lined separation chambers were pre-equilibrated with the solvent for several hours at 4°C. These were tightly sealed by using silicone rubber sealing and pressure fixation over the cover plate.

The solvent system consisted of chloroform:methanol:acetic acid: 0.9% w/v NaCl solution (50:25:8:2.5) for separation of phospholipids in the LAT assay and petrol ether:diethyl ether:acetic acid (50:50:1) for separation in the PLA_2 assay. Plates of 20 cm length were run for the LAT assay for 2 h and for the PLA_2 assay for 45 min. Lipids were localized by iodine vapour and the respective areas cut out. Radioactivity was determined in a LKB liquid scintillation counter (LKB Wallac 1217 Rack-beta) for 2 min using a toluene scintillator.

Gastric mucosal eicosanoid formation and release

Isolated gastric mucosal cells (2×10^6 cells ml^{-1}) were incubated at pH 7.4 for 60 min at 37°C. After 60 min the cells were spun down and the release of PGI_2 (measured as the stable metabolite 6-keto $PGF_{1\alpha}$) PGE_2, $PGF_{2\alpha}$ and TXA_2 (measured as the stable metabolite TXB_2) was determined in the clear supernatant fluid by radio-immunoassay according to Peskar *et al.* (1979).

Other determinations

Protein content was measured according to the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. If not otherwise stated all data are expressed as means ± s.e.mean. Results were statistically analysed by use of the *t* test for paired comparison.

Materials

Collagenase 152–245 u mg^{-1} (Sigma Munich), pronase E (70,000 p.u.k.g^{-1}; Merck, Darmstadt), bovine serum albumin (Serva, Munich), omeprazole (kindly supplied by Dr E. Carlsson, Hässle, Mölndal, Sweden), DMSO and PEG 400 (Merck), merthiolate (Sigma), Na-cholate (Dr T. Schuchardt, Munich), oleic acid (Serva), L-a-phosphatidylcholine from egg yolk (Sigma), arachidonic acid (Nu-Chek-Prep). Arachidonoyl coenzyme A was synthesized according to Reitz *et al.* (1968) and Szamel & Resch (1981) using arachidonoyl chloride (Nu-Chek-Prep) and coenzyme A (Boehringer) as substrates.

L-3-phosphatidylcholine, 1-palmitoyl-2-[1-^{14}C] oleoyl (sp. act. 57 mCi $mmol^{-1}$) and L-lyso-3-phosphatidylcholine, 1-[1-^{14}C] palmitoyl (sp. act. 61 mCi $mmol^{-1}$) from Amersham Buchler, Braunschweig, F.R.G. Prostaglandin E_2 (PGE_2), 6-keto $PGF_{1\alpha}$, $PGF_{2\alpha}$ and thromboxane B_2 (TXB_2) were purchased from Sigma, Munich. Radiolabelled prostaglandins for the radioimmunoassay [5,6,8,11,12,14,15-^{3}H]-PGE_2 (sp. act. 160 Ci $mmol^{-1}$), [5,6,8,9,11,12,14,15-^{3}H]-6-keto $PGF_{1\alpha}$ (sp. act. 170 Ci $mmol^{-1}$), [5,6,8,9,11,12,14,15-^{3}H]-TXB_2 (sp. act. 113 Ci $mmol^{-1}$) from New England Nuclear, Dreieich, F.R.G. and [5,6,8,9,11,12,14,15-^{3}H]-$PGF_{2\alpha}$ (sp. act. 180 Ci $mmol^{-1}$) from Amersham Buchler, Braunschweig, F.R.G.

The antibody against PGE_2 was raised in our laboratory according to Salmon (1978). The antibodies against 6-keto $PGF_{1\alpha}$ and TXB_2 were a generous gift from Prof. Gemsa, Marburg, F.R.G. The antibody against $PGF_{2\alpha}$ was purchased from Paesel GmbH & Co, Frankfurt, F.R.G. The sensitivities of the assays (detection limits defined as 10% displacement of tracer) per sample (in pg 200 μl^{-1}) were as follows: PGE_2, 10; 6-keto $PGF_{1\alpha}$, 2; $PGF_{2\alpha}$, 2; TXB_2, 2. The intra- and inter-assay coefficients of variation were (in %): PGE_2, 8/9; 6-keto $PGF_{1\alpha}$, 7/8; $PGF_{2\alpha}$, 9/9 and TXB_2, 9/13. Goat antiserum to rabbit-gamma-globulin was purchased from Calbiochem (Behring Diagnostics, La Jolla CA 92037).

Results

Effect of omeprazole on lysophosphatide acyltransferase

Basal LAT activity in gastric mucosal cells was 49 ± 9 nmol mg^{-1} protein min^{-1} and did not significantly differ from that in isolated and enriched parietal cells (46 ± 5 nmol mg^{-1} protein min^{-1}) and that in mucous cells (42 ± 8 nmol mg^{-1} protein min^{-1}). Omeprazole dissolved in DMSO pH 2 inhibited LAT in a concentration-dependent manner as shown in Figure 1 (IC_{50} values: gastric cells 12.9 ± 2.5, parietal cells 16.3 ± 1.4 and mucous cells 9.4 ± 1.4 μM). A solution of omeprazole in DMSO pH 7 shifted the concentration-response curves to the right (IC_{50} values: gastric cells 19.8 ± 1.1 μM, NS; parietal cells 27.8 ± 2 μM, $P < 0.01$; mucous cells 19 ± 1.7 μM, $P < 0.05$).

LAT was assayed at pH 6. At pH 6 omeprazole is partially degraded as shown by Wallmark (1986). Therefore, in one set of experiments LAT was assayed at the physiological pH 7.4 which is higher than its pH optimum. LAT activity in gastric cells was only 16.7% of that at pH 6 (8.2 ± 3.4 nmol mg^{-1} protein min^{-1}, $n = 6$ experiments). At this pH omeprazole dissolved in DMSO pH 7 had no inhibitory effect on LAT.

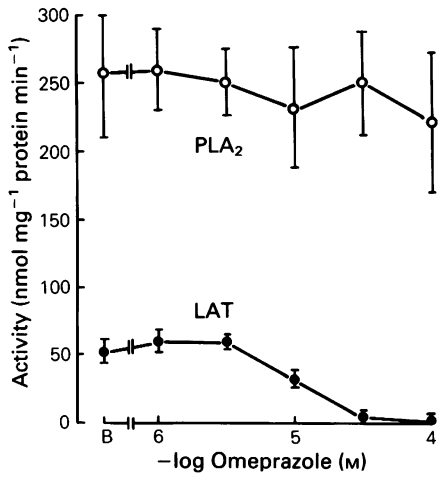

Figure 1 Effect of omeprazole on guinea-pig gastric mucosal phospholipase A_2 (○, PLA_2) and lysophosphatide acyltransferase (●, LAT). Vertical lines show s.e.mean; B = basal. $n = 3$ preparations. IC_{50} for effect of omeprazole on LAT = 12.9 ± 2.5 μM.

Effect of omeprazole on phospholipase A_2

Basal PLA_2 activity was 258 ± 47 (gastric cells), 255 ± 52 (parietal cells) and 284 ± 16 (mucous cells) nmol mg^{-1} protein min^{-1}. In no system and at no pH did omeprazole, up to 100 μM have any effect (Figure 1).

Effect of merthiolate on lysophosphatide acyltransferase phospholipase A_2

The SH-reagent merthiolate inhibited LAT activity in a concentration-dependent manner (IC_{50} values: gastric cells 1.7 ± 0.3, parietal cells 1.6 ± 0.3 and mucous cells 1.4 ± 0.2 μM, $n = 3$ preparations for each cell type). It had no effect on PLA_2 in gastric cells ($n = 2$, data not shown).

Eicosanoid formation in guinea-pig gastric mucosal cells

The gastric cell mixture continuously produced PGI_2, PGE_2, $PGF_{2\alpha}$ and TXA_2 during the 60 min of incubation. The main prostaglandin released (in pg 10^{-6} cells h^{-1}) was PGI_2 (37.4 ± 12.8) followed by PGE_2

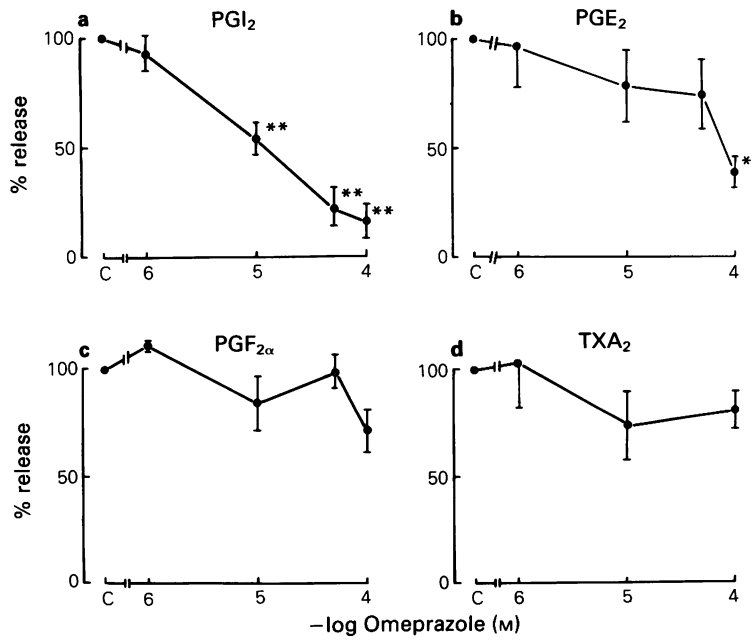

Figure 2 Effect of omeprazole in polyethylene glycol pH 2 on spontaneous release of (a) prostaglandin I_2 (PGI_2, $n = 5$), (b) PGE_2 ($n = 3$), (c) $PGF_{2\alpha}$ ($n = 2$) and (d) thromboxane A_2 (TXA_2, $n = 5$). Control (C) eicosanoid release was set as 100%. Asterisks indicate significant differences, $*P < 0.05$, $**P < 0.01$. IC_{50} for effect of omeprazole on PGI_2 release = 14.3 ± 4.8 μM.

(31.8 ± 8.5). Smaller amounts of $PGF_{2\alpha}$ (8.7 ± 2.8) and TXA_2 (11 ± 1.7) were formed.

Effect of omeprazole on spontaneous eicosanoid release

The effect of omeprazole dissolved in PEG pH 2 on spontaneous eicosanoid release from guinea-pig gastric mucosal cells is shown in Figure 2. Omeprazole inhibited PGI_2 release with an IC_{50} of 14.3 ± 4.8 μM. PGE_2 release was significantly inhibited by 60% in the presence of 100 μM omeprazole. Up to 100 μM omeprazole produced no significant changes in the formation of $PGF_{2\alpha}$ and TXA_2. PEG pH 2 itself had no effect on spontaneous eicosanoid release.

Effect of pH on spontaneous release of PGI_2 and PGE_2

For these experiments omeprazole was dissolved either in PEG pH 2 or pH 7. The concentration-response curve for omeprazole in PEG pH 2 was similar to that in Figure 2. However, the solution of PEG pH 7 only at the highest concentration (100 μM) resulted in an insignificant inhibition of PGI_2 release by 40% compared to control values (Figure 3). A solution of omeprazole in PEG pH 7 caused no significant change in the formation of PGE_2.

Effect of merthiolate on spontaneous release by PGI_2

Merthiolate inhibited spontaneous release of PGI_2 in a concentration-dependent manner with an IC_{50} of 10.1 ± 2.4 μM.

Discussion

Our data have shown that acid-activated omeprazole inhibits LAT in a concentration-dependent manner without having an effect on PLA_2. In contrast to that which was seen in peritoneal macrophages (Körner *et al.*, 1984) there was no rise in the formation and release of PGI_2, PGE_2, $PGF_{2\alpha}$ and TXA_2. In fact there was a concentration-dependent inhibition of the release of PGI_2 and PGE_2 without any effect on $PGF_{2\alpha}$ and TXA_2. The reason for this may be twofold. First, peritoneal macrophages had a relatively low activity of PLA_2 compared to that of LAT (ratio 1:50) (Körner *et al.*, 1984). The authors concluded that the rate of prostanoid synthesis from endogenously released arachidonic acid is controlled by LAT, which apparently regulates the substrate availability for the cyclooxygenase and probably lipoxygenase pathways, but not by PLA_2. However, in guinea-pig gastric mucosal cells the ratio PLA_2/LAT is inverse (5:1) indicating that the substrate availability is controlled by PLA_2 and that inhibition of LAT by omeprazole has no significant stimulatory effect on the amount of arachidonic acid available for prostanoid synthesis. Therefore, it is logical that, in contrast to the results presented by Körner *et al.* (1984) for macrophages, we found an inhibition of eicosanoid release in gastric mucosal cells. Second, De Witt *et al.* (1983) and Ullrich & Graf (1984) have shown that prostacyclin synthase is a cytochrome P450-like enzyme with a characteristic heme-thiolate catalytic centre. This thiol structure might explain the inhibitory effect of omeprazole and merthiolate. A possible explanation for the inhibitory effect of omeprazole in high concentrations on PGE_2 formation could be provided by the results of Hamberg & Samuelsson (1973); they found that in sheep vesicular glands conversion of the endoperoxide into PGE_2 was stimulated by reduced glutathione but suppressed by SH-reagents like *p*-mercuribenzoate and N-ethylmaleimide. The unchanged formation of thromboxane A_2 was in accordance with the results of Hammarström & Falardeau (1977) who showed that thromboxane synthase in human platelets was insensitive to sulphydryl reagents and thiols.

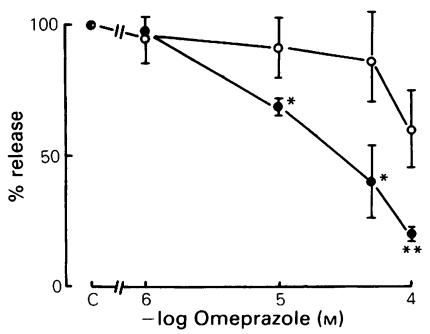

Figure 3 Effect of omeprazole dissolved in polyethylene glycol (PEG) pH 2 (●) and PEG pH 7 (○) on spontaneous prostacyclin (PGI_2) release from gastric mucosal cells. $n = 3$. Asterisks indicate significant differences, $*P < 0.05$, $**P < 0.01$.

Significant changes in PGI_2 and PGE_2 release as with LAT were only seen with acid-activated omeprazole. Such an activation probably only occurs in the tubulovesicles of the parietal cells (Beil & Sewing, 1985). Therefore, it is unlikely that omeprazole under therapeutic conditions has an effect on prostacyclin synthase and PGE_2 isomerase and LAT in other tissues. This is important since prostacyclin is the main prostanoid generated by blood vessels and a potent vasodilator (Moncada & Vane, 1978). In agreement with that view Sehayek *et al.* (1985) found no effect of omeprazole on hepatic flow in the artificially perfused rat liver.

In summary, our data indicate that if omeprazole, apart from its antisecretory properties, has an additional protective effect it is not related to an

increased synthesis of prostaglandins although acid-activated omeprazole interacts with enzymes of the prostaglandin cascade.

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