Prostaglandin protects against bile salt induced increases in proton permeation of duodenal brush border membrane

D Zhao, B H Hirst

Abstract

A direct protective action of prostaglandin on luminal cell membranes was investigated by preincubating rabbit duodenal brush border membrane vesicles with prostaglandin E_2 (PGE_2) before incubation with bile salts. Membrane perturbation was assessed by measuring the net proton permeability (P_{net}). Bile salts (deoxycholate, glycodeoxycholate, and taurodeoxycholate; 0.1–1.0 mmol/l) resulted in concentration dependent increases in P_{net} : from (mean (SE)) 5.42 (0.17) ($n=20$) to 8.44 (0.24) $\times 10^{-4}$ ($n=13$) cm/s with 0.5 mmol/l deoxycholate. PGE_2 , 10^{-8} – 10^{-6} mol/l, when added alone had no effect on P_{net} : 5.41 (0.21) $\times 10^{-4}$ ($n=14$) cm/s with 10^{-6} M PGE_2 . When duodenal brush border membranes were preincubated with PGE_2 , 10^{-7} – 10^{-6} mol/l, however, the bile salt induced increase in P_{net} was significantly reduced: 7.22 (0.18) $\times 10^{-4}$ ($n=13$) cm/s with 10^{-7} mol/l PGE_2 and 0.5 mmol/l deoxycholate. These findings indicate that PGE_2 exerts a direct protective action on duodenal luminal membranes.

The mechanism(s) by which prostaglandins protect the gastrointestinal mucosa against damaging agents^{1,2} is still unclear, despite considerable scientific effort. Prostaglandins show many activities which may contribute towards protective mechanisms.^{3–6} At the vascular level maintenance of endothelial integrity and mucosal blood flow is a recognised gastric protective mechanism.^{7–9} Recent evidence also points to a direct cellular protective action.^{10–15}

We have established the use of apical membrane vesicles isolated from the upper gastrointestinal tract as model systems for studying the mechanism of action of several damaging agents, including ethanol and bile salts.^{16–19} Increased apical membrane permeability to protons is a sensitive indicator of the action of these damaging agents.^{18,19} In the present study we have investigated whether prostaglandin E_2 (PGE_2) has a direct protective action on upper gastrointestinal luminal membranes by studying their ability to reduce the increase in proton permeability induced by bile salts in rabbit duodenal brush border membrane vesicles.

Methods

PREPARATIVE AND ANALYTICAL METHODS

Duodenal brush border membrane vesicles were prepared from young New Zealand white rabbits by a slight modification of the method of Kessler

et al.,²⁰ as described previously.^{16–19} The enclosed volume of the vesicles was determined as the retention of [¹⁴C]glucose at equilibrium (45 min) in a solution containing 150 mmol/l NaCl and 0.2 mmol/l glucose, by a rapid filtration technique, and expressed as μ l/mg membrane protein.^{16–19} Protein was determined by the method of Bradford²¹ using γ globulin (Cohn fraction IV) as standard.

The net H^+/OH^- permeability (P_{net}) of brush border membrane vesicles was determined as described by Wilkes *et al.*¹⁸ Membrane vesicles were incubated on ice for 25 minutes in a pH 6.5 solution (150 mmol/l K^+ gluconate, 10 mmol/l hydroxyethylpiperazine-ethanesulphonic acid (HEPES)/Tris) containing 4 μ mol/l valinomycin (to voltage-clamp the vesicles), with varying concentrations of bile salts or PGE_2 , or both. Proton permeation was monitored by diluting vesicles in 150 mmol/l K^+ gluconate, 6 μ mol/l acridine orange, 10 mmol/l HEPES/Tris at pH 8.0. The fluorescence signal from the acridine

Figure 1: The effect of PGE_2 on bile salt induced increase in proton permeation in duodenal brush border membrane vesicles. On addition of vesicles equilibrated at pH 6.5 to a solution containing acridine orange, at pH 8.0, fluorescence (λ_{ex} 490 nm, λ_{em} 522 nm) quenching is observed due to accumulation of acridine orange into the acidic intravesicular compartment. As the pH gradient is dissipated, quenching is reduced. Addition of nigericin, a K^+/H^+ ionophore, completely dissipates the pH gradient. The traces illustrate experiments with vesicles preincubated (a) under control conditions, (b) with PGE_2 , 10^{-7} mol/l, (c) with deoxycholate 0.5 mmol/l, and (d) with PGE_2 , 10^{-7} mol/l, plus deoxycholate 0.5 mmol/l. dBBM = duodenal brush border membrane vesicles.

Department of
Physiological Sciences,
University of Newcastle
upon Tyne Medical
School, Newcastle upon
Tyne NE2 4HH
D Zhao
B H Hirst

Correspondence to:
Dr Barry H Hirst.

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orange (excitation 490 nm; emission 522 nm) was monitored in a Perkin Elmer LS-5 spectrofluorimeter fitted with a cuvette holder maintained at 20°C. The rate of permeation of protons out of the vesicles follows simple first order kinetics,¹⁸ and was quantified by fitting the recovery of acridine orange fluorescence quenching as a function of time with a non-linear regression analysis procedure (Enzfitter, Elsevier Biosoft).¹⁹ The net flux of proton and hydroxide ions (P_{net}) was calculated from the exponential time constant for recovery of acridine orange fluorescence (τ) by

$$P_{\text{net}} = P_{\text{H}} + P_{\text{OH}} = \frac{r \cdot \beta_{\text{end}} + \beta_{\text{ex}}}{3\tau[H^+]_i \cdot \ln 10},$$

using the constants derived by Wilkes *et al.*,¹⁸ and where r is the mean vesicle radius (0.1 μm), β_{end} the endogenous buffer capacity of the vesicles (45 mmol/l/pH unit), β_{ex} the external buffer capacity of the solution, and $[H^+]_i$ the concentration of free protons within the vesicle interior.

BILE SALT AND PROSTAGLANDIN E_2 TREATMENT

Deoxycholate, glycodeoxycholate, and taurodeoxycholate acid (Steraloids) and PGE_2 (Sigma) were made up fresh each day and final pH adjusted after sonication. The vesicles were preincubated with PGE_2 for five minutes before the addition of bile salts and incubated for a further 20 minutes before analysis.¹⁹ Appropriate controls without bile salts or without PGE_2 were included.

STATISTICAL ANALYSIS

Significance of difference between mean values was investigated by analysis of variance followed by Student's t test. Significance was set at $p < 0.05$, and results are expressed as mean (SE) (n).

Results

EFFECTS OF PGE_2 AND BILE SALTS ON PROTON PERMEATION IN DUODENAL BRUSH BORDER MEMBRANE VESICLES

Under control condition without bile salts or PGE_2 , the mean net proton/hydroxide permeability coefficient (P_{net}) was (mean (SE)) $5.42 (0.17) \times 10^{-4}$ ($n=20$) cm/s . The rate of proton permeation, as determined by the rate of recovery of acridine orange fluorescence quenching, was accelerated by the bile salts (Fig 1). P_{net} was increased in a concentration dependent manner by approximately 32%, 56%, and 98% with deoxycholate at 0.1, 0.5, and 1.0 mmol/l, respectively (Fig 2). Similar increases in proton permeability were observed with glycodeoxycholate (Fig 3) and taurodeoxycholate (Fig 4). The unconjugated deoxycholate tended to have the most pronounced effect in increasing proton permeability.

PGE_2 , 10^{-8} – 10^{-6} mol/l, had no effect of its own on the proton permeability of duodenal brush border membrane (Fig 1). P_{net} was (mean (SE)) $5.30 (0.16)$ ($n=12$), $5.38 (0.22)$ ($n=9$), and $5.41 (0.21)$ ($n=14$) $\times 10^{-4}$ cm/s in the presence of PGE_2 ,

Figure 2: The effect of PGE_2 on deoxycholate induced increase in proton permeation in duodenal brush border membrane vesicles. Proton permeability P_{net} is plotted against the concentration of deoxycholate with and without ($\circ$) pretreatment with PGE_2 : 10^{-8} ($\bullet$), 10^{-7} ($\blacktriangle$), or 10^{-6} mol/l ($\blacksquare$). Results are plotted as mean values with error bars of 1 SE, $n=8-20$; * $p < 0.05$ compared with values without PGE_2 .

10^{-8} , 10^{-7} , and 10^{-6} mol/l, respectively (Figs 2 and 3).

Preincubation of duodenal brush border membrane with PGE_2 , 10^{-8} mol/l, for five minutes before the addition of any of the bile salts only significantly reduced the ability of taurodeoxycholate, 0.5 mmol/l, to increase P_{net} (Fig 4). At higher concentrations, however, PGE_2 significantly reduced the bile salt induced increases in P_{net} . The effect of PGE_2 , 10^{-7} mol/l, on the rate of recovery of acridine orange fluorescence quenching in the presence of 0.5 mmol/l deoxycholate is shown in Figure 1. PGE_2 was most effective against taurodeoxycholate induced increases in P_{net} (Fig 4) and least effective against glycodeoxycholate (Fig 3).

EFFECTS OF PGE_2 AND BILE SALTS ON ENCLOSED VOLUME OF DUODENAL BRUSH BORDER MEMBRANE VESICLES

The enclosed volume of untreated duodenal brush border membrane vesicles was (mean (SE)) $0.533 (0.011)$ ($n=30$) $\mu\text{l/mg}$ protein. PGE_2 , 10^{-8} – 10^{-6} mol/l, did not influence this value; $0.494 (0.015)$ ($n=10$), $0.508 (0.016)$ ($n=8$), and $0.517 (0.022)$ ($n=16$) $\mu\text{l/mg}$ protein

Figure 3: The effect of PGE_2 on glycodeoxycholate induced increase in proton permeation in duodenal brush border membrane vesicles. Proton permeability P_{net} is plotted against the concentration of glycodeoxycholate with and without ($\circ$) pretreatment with PGE_2 : 10^{-8} ($\bullet$), 10^{-7} ($\blacktriangle$), or 10^{-6} mol/l ($\blacksquare$). Results are plotted as mean values with error bars of 1 SE, $n=8-20$; * $p < 0.05$ compared with values without PGE_2 .

Figure 4: The effect of PGE_2 on taurodeoxycholate induced increase in proton permeability in duodenal brush border membrane vesicles. Proton permeability P_{net} is plotted against the concentration of taurodeoxycholate with and without ($\circ$) pretreatment with PGE_2 : 10^{-8} ($\bullet$), 10^{-7} ($\blacktriangle$), or 10^{-6} mol/l ($\blacksquare$). Results are plotted as mean values with error bars of 1 SE, $n=8-20$; * $p<0.05$ compared with values without PGE_2 .

with PGE_2 10^{-8} , 10^{-7} , and 10^{-6} , respectively. The three bile salts decreased vesicular enclosed volume in a concentration dependent manner (Fig 5). Preincubation of duodenal brush border membrane with PGE_2 , 10^{-6} mol/l, inhibited the decrease in enclosed volume induced by the three bile salts at a concentration of 0.1 mmol/l. PGE_2 at the lower concentrations had no consistent effect on the bile salt induced reductions in enclosed volume. The exception was PGE_2 at 10^{-8} and 10^{-7} mol/l, which significantly enhanced the reduction in enclosed volume induced by unconjugated deoxycholate (Fig 5).

Discussion

The present study provides evidence that prostaglandin exerts a direct protective action on duodenal membranes. In particular, PGE_2 reduced the bile salt induced increase in membrane proton permeability. Increased proton permeability in the presence of bile salts is likely to be an early event in their damaging action, and is observed with relatively low concentrations.¹⁹ Thus, this protective effect of PGE_2 is likely to be important in maintaining normal duodenal integrity. PGE_2 at 10^{-6} mol/l also had a lesser effect in preventing bile salt induced reduction in membrane vesicle integrity, as shown in the reduction in enclosed volume. Paradoxically, at the two lower concentrations PGE_2 enhanced the damaging effects of unconjugated deoxycholate on membrane integrity. The reason for this last effect remains obscure.

Direct membrane protective effects of prostaglandin have been implied in other studies. A high concentration of PGE_2 , 10^{-3} mol/l, improved cell viability after taurocholate treatment in isolated rat gastric mucosal cells.¹⁰ Similar protective effects have been observed with lower concentrations of 16,16-dimethyl PGE_2 , 10^{-8} – 10^{-6} mol/l, against injury induced by taurocholate, ethanol, or indomethacin in rat gastric mucosal cells in culture, human isolated gastric glands, and guinea pig isolated peptic cells.¹¹⁻¹⁵ These last studies provide considerable evidence for a direct protective action of prostaglandin on gastric cells, although this conclusion has been disputed by others.²² In the liver prostacyclin prevented lysosomal and cytoplasmic enzyme release in response to hypoxic damage, suggesting a membrane stabilising effect.²³ Low concentrations of PGE_2 decrease erythrocyte membrane deformability.^{24,25} In isolated gastric mucosal cell membranes high concentrations of taurocholate, 100 mmol/l, increased the polar part of the electron spin resonance of 16-doxylstearic acid spin label, consistent with disintegration of the membranes and formation of micellar aggregates with taurocholate.²⁶ Similar membrane dissolution has been observed with deoxycholate salts at 5 mmol/l in duodenal brush border membrane.¹⁹ The effect of taurocholate on the gastric mucosal membranes was completely prevented by preincubation with 25 nmol PGE_2 .²⁶ These results, considered with our own, perhaps point to some generalised membrane stabilising effects of prostaglandin, such as stabilising polar phospholipid head groups, and hence ameliorating the membrane perturbing action of damaging

Figure 5: The effect of PGE_2 on bile salt induced decrease in duodenal brush border membrane vesicle enclosed volume. Enclosed volume (^3H glucose space at equilibrium) is plotted against the concentration of deoxycholate, glycodeoxycholate, and taurodeoxycholate, with and without ($\circ$) pretreatment with PGE_2 : 10^{-8} ($\bullet$), 10^{-7} ($\blacktriangle$), or 10^{-6} mol/l ($\blacksquare$). Results are plotted as mean values with error bars of 1 SE, $n=8-30$; * $p<0.05$ compared with values without PGE_2 .

agents. In erythrocytes, however, 16,16-dimethyl PGE₂ was unable to alter alcohol or aspirin induced haemolysis and slightly increased haemolysis when administered alone.²⁷ This last observation may be related to the increased fragility of duodenal brush border membrane vesicle observed with the lower concentrations of PGE₂ when administered with bile salts (Fig 5).

The effects of prostaglandin in the gastrointestinal tract are diverse, with many actions which may be categorised as protective in nature. An impaired ability of the human duodenal mucosa in duodenal ulcer disease to synthesise prostaglandin has been noted in some studies.^{28, 29} Ahlquist *et al.*, however, reported no difference in basal prostaglandin synthesis in duodenal ulcer patients and normal controls.³⁰ In contrast, the normal increased prostaglandin synthesis in response to a meal was attenuated in duodenal ulcer disease, suggesting the mucosa of these patients has an impaired response to an acid load.³⁰ The ability of PGE₂ to reduce bile salt induced increases in duodenal membrane acid permeation may be one important and direct physiological mechanism for duodenal mucosal protection.

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