Prostaglandin E₂ formation by the gall bladder in experimental cholecystitis

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SUMMARY Both experimental cholecystitis and luminal distension inhibit fluid absorption and stimulate motor activity in the gall bladder. These functional alterations are mimicked by exogenous prostaglandins (PGs) and inhibited by potent cyclooxygenase inhibitors, but direct evidence of a primary role of endogenous PGs is not available. Therefore, experiments in the cat were carried out in which the effects of lyso-phosphatidylcholine (lysoPC; 0.5–2.0 mmol/l), implantation of cholesterol stones, and raised intraluminal pressure in the gall bladder lumen were assessed. The gall bladder was perfused *in vivo* at a constant rate by a buffer solution. PGE₂ was determined in the effluent by a radioimmunological method validated by gas chromatography-mass spectrometry. PGE₂ output was markedly ($p < 0.01$) raised (13.9 ± 2.6 vs 1.1 ± 0.5 ng/h; $n = 10$) during lysoPC perfusions and this response was inhibited by 66% ($p < 0.02$) after indomethacin administration (2 mg/kg *iv*). A significant ($p < 0.05$) increase in PGE₂ output occurred six weeks after implantation of gall stones (3.7 ± 1.5 ng/h; $n = 6$) and in response to distension of the normal gall bladder wall (3.6 ± 1.2 ng/h; $n = 6$). These findings support the theory that PGs play an important pathophysiologic role in biliary tract disease.

Prostaglandins (PGs) are easily formed by various tissues in response to chemical or mechanical injury,¹ and are generally considered important mediators of the inflammatory response as well as pain reactions.² Furthermore, they have pronounced effects on normal gall bladder function. Thus, PGs of the E type have been shown to inhibit fluid absorption, induce fluid secretion, and cause muscle contraction in the gall bladder *in vitro*^{3,4} and *in vivo*.⁵

Obstruction and distention of the gall bladder, presence of calculi in the gall bladder lumen, and increased proportions of lysoPC in gall bladder bile are all factors suggested to be of importance in the aetiology of acute cholecystitis.⁶ In animal experiments, these noxious factors cause functional alterations similar to those shown in response to exogenous PGE₂.⁷⁻⁹ These alterations, as well as biliary pain in human cholecystitis,¹⁰ are inhibited by

potent cyclooxygenase inhibitors, such as indomethacin, which provides indirect evidence for the notion that PGs are involved in the pathogenesis of cholecystitis and biliary pain. Although gall bladder tissue has been shown to synthesise PGs,^{3,11-13} direct evidence of a primary pathophysiologic role of PGs in the biliary tract is still missing.

The present study was designed to evaluate whether experimental cholecystitis or distension of the gall bladder wall in the absence of inflammation might influence local formation of endogenous PGs. As quantification of the 'overflow' of PGs from the epithelium into the gall bladder lumen should provide the most reliable index of the balance between local PG formation and degradation *in vivo*,¹⁴ we used a perfusion technique for determination of gall bladder PGE₂ output in the cat.

Methods

EXPERIMENTAL PROCEDURES

Experiments were carried out on 22 cats of either sex (10 males, 12 females). The animals were

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deprived of food for 24 hours before the experiments but allowed free access to water. After cannulating the gall bladder *in situ* it was continuously perfused by a roller pump at a rate of 10 ml/h in an open perfusion system allowing adjustment of the intraluminal pressure as previously described.⁷ The perfusate was an electrolyte solution (mmol/l: 135 Na⁺, 5.0 K⁺, 105 Cl⁻, 35 HCO₃⁻). Experimental cholecystitis was induced by implantation of human gall stones into the duct ligated gall bladder six weeks before the experiments,⁸ or by lysoPC, prepared from egg yolk phosphatidylcholine (PC), which was added to the perfusate during the perfusion procedure.⁹

EXPERIMENTAL DESIGN

Determination of total PGE₂ output into the gall bladder lumen was based on the perfusion rate and the accumulation of PGs in samples of the effluent after a 60-minute perfusion period.

(1) During control conditions PGE₂ output was determined in 16 animals. (2) In 10 animals the perfusion was continued for another two 60-minute periods after the addition of lysoPC (final concentration mmol/l: 0.5, n=3; 1.0, n=4; 2.0, n=3). Phosphate buffered indomethacin (Confortid^R, Dumex Ltd., Copenhagen, Denmark) 2 mg/kg dissolved in sterile water was given intravenously before the second experimental period. PGE₂ output was determined in all animals. (3) In six animals the control period was followed by a 60-minute period during which the gall bladder was distended by increasing the intraluminal pressure to 50 cm H₂O. PGE₂ output was studied in both periods. (4) Gall bladder perfusions were carried out in six animals with experimental cholecystitis after implantation of gall stones. PGE₂ output was studied during this test period. (5) Finally PGE₂ was assayed in three control samples of perfusate containing lysoPC (0.5, 1.0 and 2.0 mmol/l respectively).

ANALYTICAL PROCEDURES

Immunoreactive PGE₂ was measured, as previously described in detail,^{15,16} by a radioimmunological method validated by gas chromatography-mass spectrometry.¹⁷ The method includes purification by extraction with ethylacetate/cyclohexane=1:1 and chromatography on microcolumns of Sephadex^R LH-20 (Pharmacia; Uppsala, Sweden) before carrying out the radioimmunoassay itself on the eluate fraction containing PGE₂. The values were expressed as ng/h.

HISTOLOGIC EXAMINATIONS

Gall bladder specimens from representative experi-

ments were fixed in 10% formaldehyde solution. Sections stained with haematoxylin-eosin and haematoxylin – van Gieson's stain were examined by microscopy and evaluated by an independent pathologist.

STATISTICAL METHODS

The results were given as means ($\pm$ SEM), and the data analysed both by Student's *t* test and Wilcoxon's tests for paired or unpaired variates. The highest *p* value was given and a *p* value less than 0.05 was considered significant.

Results

MORPHOLOGIC CHANGES

Gall bladders exposed to lysoPC revealed acute inflammatory lesions as oedema and mucosal bleeding, and microscopic examination showed infiltration of inflammatory cells. Gall bladders exposed to high intraluminal pressure showed no visible lesions and were normal by histologic appearance. Obstructed gall bladders containing gall stones revealed chronic lesions in addition to acute inflammatory changes. The wall was markedly thickened by fibrosis, and the mucosa was invaded by inflammatory cells.

PGE₂ OUTPUT INTO THE GALL BLADDER LUMEN
PGE₂ output during control conditions averaged ng/h 1.3 ± 0.4 (n=16). Figures 1 and 2 illustrate, that

Fig. 1 Luminal output of PGE₂ during *in vivo* perfusion of the gall bladder with control solution (A) before and (B) after gall bladder distension, in experimental cholecystitis (C) after implantation of cholesterol stones, or during perfusion with (D) lysophosphatidylcholine (lysoPC; 0.5–2.0 mmol/l). Values are means ($\pm$ SEM).

Fig. 2 Luminal output of PGE₂ during in vivo perfusion of the gall bladder with control solution (basal) and lysophosphatidylcholine (lysoPC 0.5–2.0 mmol/l) before and (indo) after administration of indomethacin (2 mg/kg iv). Values are means (±SEM).

the luminal output of PGE₂ increased significantly ($p < 0.05$), both in experimental cholecystitis and in response to mechanical stimulation (distension) of the gall bladder, compared with control conditions.

The most prominent response was observed during perfusion with lysoPC, which caused a 12-fold increase in PGE₂ output ($13.9 \text{ ng/h} \pm 2.6$ vs $1.1 \text{ ng/h} \pm 0.5$; $n=10$; $p < 0.01$). The luminal release of PGE₂ in these experiments was inhibited by 66% after indomethacin administration, but did not reach the normal range (Fig. 2), not even during perfusion with low (0.5 mmol/l) concentration of lysoPC.

Inflammation of the duct ligated gall bladder, induced by implantation of gall stones, was associated with a three-fold increase ($p < 0.05$) in PGE₂ output ($3.7 \text{ ng/h} \pm 1.5$; $n=6$) compared with control conditions (see Fig. 1). A similar response in PGE₂ output was observed in response to distension of the gall bladder wall ($3.6 \text{ ng/h} \pm 1.2$; $n=6$, $p < 0.05$) as shown in Figure 1. No sex difference in PG output was detected and no PGE₂ was found in control assays of perfusate containing lysoPC.

Discussion

Determination of PGs in biologic material encounters special methodologic problems, because PGs are usually not stored, but rapidly synthesised immediately before their release, and because they have an ultra short half life in the circulation.¹ Even more important are the difficulties inherent in the choice of an experimental design, which prevents artificial stimulation of PG biosynthesis *in vitro* – for example, by aggregating platelets or by the inevit-

able manipulation with tissue specimens, and the obstacles to interpretation of the results, because the origin of endogenous PGs measured is poorly defined.¹⁴ Considering the named problems, determination of the amount of PGs released by the epithelium into the gall bladder lumen – that is, the 'overflow' of PGs, appears *a priori* most attractive, because this 'atraumatic' approach permits a quantitative comparison of estimates of the balance between local *in vivo* PG synthesis and degradation measured during control and experimental conditions – the capacity of enzymatic degradation being unknown.

In addition to acute (distension) and chronic (implantation of gall stones) mechanical stimulation of the gall bladder epithelium we used chemical stimulation with lysoPC, which is a hydrolytic product of PC. This was chosen because it is a normal constituent of bile and increased proportions of lysoPC in gall bladder bile from patients with acute cholecystitis has been reported.^{18,19} The substance, which has cytolytic and membrane perturbing properties,²⁰ is capable of inducing acute inflammation of the gall bladder in experimental animals.^{9,21,22} The lysoPC concentration range used for gall bladder perfusion in the present study equalled that observed in human cholecystitis.^{18,19} LysoPC perfusions were carried out using an electrolyte solution, however, rather than native bile, to eliminate the possibility that some small amount of bile acid, remaining in the eluate fraction containing PGs, would cross-react non-specifically with antibodies against PGs during the analytical procedure. While there is no doubt that increased amounts of PGE₂, measured during experimental conditions, originate in the gall bladder, invading inflammatory cells are probably an important source of PG formation in acute and chronic cholecystitis. In contrast, the significantly increased luminal PGE₂ release in response to distension of the gall bladder wall in the absence of inflammation is most likely brought about by the epithelial cells lining the lumen.

The named procedures are known to cause alterations in gall bladder function similar to those seen in response to exogenous PGE₂, and which are reversible by indomethacin. Furthermore, from studies in man it is known that cyclooxygenase inhibitors relieve biliary pain,¹⁰ and lower the high intraluminal gall bladder pressure seen in patients with acute cholecystitis.²³ In the light of this knowledge, the present findings strongly support the theory that PGs play a decisive role in the pathophysiology of biliary tract disease.

PGE₂ output was inhibited by indomethacin, which suggests that the 'overflow' of PGs from the

gall bladder epithelium parallels the local production, and consequently mucosal PGE₂ concentrations. Thus, the daily urinary excretion of PG metabolites in man is reduced to an average of 30% during the first day of treatment with conventional doses of indomethacin,²⁴ which compares well with the finding of a 66% inhibition of PGE₂ output by indomethacin in the present study.

In conclusion, acute and chronic cholecystitis, as well as mechanical stimulation of the gall bladder, is accompanied by excess production of endogenous PGE₂. Although our data are correlative in nature, they suggest that PGs play an important pathophysiological role in biliary tract disease as mediators of inflammation, gall bladder dysfunction, and biliary pain. Therefore, the demonstration of increased gall bladder PGE₂ output, provides a rationale for symptomatic treatment of acute cholecystitis with potent cyclooxygenase inhibitors.

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