Increased gall-bladder prostanoid synthesis after bile-duct ligation in the rabbit is secondary to new enzyme formation

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Ligation of the common bile duct (BDL) in the male rabbit resulted in increased gall-bladder microsomal total cyclo-oxygenase activity with prostaglandin E_2 (PGE_2) and 6-oxoprostaglandin $F_{1\alpha}$ [6-oxo-PGF $_{1\alpha}$, stable metabolite of prostaglandin I_2 (PGI $_2$; prostacyclin)] as the major prostanoids synthesized after 24 and 72 h. Kinetic analysis of gall-bladder microsomal membrane fractions incubated with increasing levels of [^{14}C]arachidonic acid indicated that BDL for 24 and 72 h did not change substrate affinity (apparent K_m) but markedly increased the rate of conversion (apparent V_{max}) suggesting the presence of more total enzyme responsible for synthesis of 6-oxo-PGF $_{1\alpha}$ and PGE_2 . BDL for 24 and 72 h significantly increased gall-bladder tissue slice basal release of 6-oxo-PGF $_{1\alpha}$, but not PGE_2 , when compared with the controls. Gall-bladder slice release of PGE_2 was 3-fold less than 6-oxo-PGF $_{1\alpha}$ in the control gall-bladder slices. Immunoblot analysis of 72 h BDL gall-bladder microsomal membrane fractions showed a slight increase in cyclo-oxygenase content and a 5-fold increase in the content of prostacyclin synthase as compared with the control. These data suggest that the BDL-stimulated total gall-bladder cyclo-oxygenase activity was the result of an increase in the level of specific prostaglandin-synthetic enzymes, in particular prostacyclin synthase, and not from a change in enzyme affinity.

INTRODUCTION

The normal rabbit gall-bladder synthesizes modest amounts of endogenous prostanoids. Several authors have hypothesized that acute inflammation of the gall-bladder stimulates increased gall-bladder prostanoid synthesis, which mediates biliary colic and mucosal injury [1]. We recently described a model of common-bile-duct ligation (BDL) in the rabbit as a new model of early acute gall-bladder inflammation. BDL for 1–3 days in the male rabbit produced both progressive histological changes of acute cholecystitis and exaggerated gall-bladder microsomal prostanoid synthesis compared with sham-operated controls [2–7]. Gall-bladder microsomal membrane fractions prepared from rabbits treated with BDL for 6, 24 and 72 h exhibited a marked increase in total cyclo-oxygenase activity, with prostaglandins I_2 (prostacyclin) and E_2 (PGI $_2$ and PGE_2) as the major prostanoids synthesized [3–7]. Although not specifically addressed, the increased gall-bladder microsomal cyclo-oxygenase activity after BDL could have been the result of new enzyme formation, increased enzyme efficiency or a combination of both. The presence of increased endogenous gall-bladder PGI $_2$ synthesis could have a profound influence on altering normal fluid absorption and contraction and thus contribute to gall-bladder distension and mucosal injury described during evolving acute cholecystitis [8–13].

The studies described above utilized gall-bladder microsomal membrane fractions incubated with ^{14}C -labelled arachidonic acid to examine prostanoid synthesis in the BDL model. These experiments provided insight into the effects of evolving cholecystitis on gall-bladder microsomal prostanoid synthesis, but not on whole gall-bladder tissue prostanoid synthesis and release.

The mechanism underlying exaggerated gall-bladder microsomal synthesis of PGI $_2$ and PGE_2 described after rabbit

BDL is the primary focus of the present study. The methodologies utilized to investigate this question will include the kinetic analysis described by Eisenthal & Cornish-Bowden and Merino [14,15] and the method of protein blot analysis [16–18]. A secondary focus of the present paper is to utilize intact gall-bladder tissue slices to determine if BDL in the rabbit stimulates both increased prostanoid synthesis and release.

MATERIALS AND METHODS

Materials

BSA was purchased from Calbiochem (San Diego, CA, U.S.A.). [^{14}C]Arachidonic acid was purchased from du Pont-NEN (Doraville, GA, U.S.A.). 3H -labelled 6-oxoprostaglandin $F_{1\alpha}$ (6-oxo-PGF $_{1\alpha}$) and PGE_2 were obtained from Amersham (Arlington Heights, IL, U.S.A.). Anti-6-oxo-PGF $_{1\alpha}$ was purchased from Dr. L. Levine, Department of Biochemistry, Brandeis University, Waltham, MA, U.S.A. Anti- PGE_2 was purchased from Advanced Magnetics (Cambridge, MA, U.S.A.). Unlabelled prostaglandin (PG) standards, L-adrenaline, EDTA, indomethacin and imidazole were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Experimental animal model

The BDL model has been previously described [2–7]. Male New Zealand White rabbits (2–3 kg) were anaesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) and maintained on halothane. The abdomen was shaved and washed with soap followed by alcohol and betadine. A midline laparotomy was performed and the common bile duct was identified just before entry into the duodenum and ligated with 4-0 silk suture (BDL) or served as a sham-operated control (BDL-sham). The gall-bladder was harvested from control rabbits and compared with those removed from animals after BDL for 24 and 72 h.

Abbreviations used: PG, prostaglandin; BDL, (common-)bile-duct ligation; PBS, phosphate-buffered saline; r.i.a., radioimmunoassay.

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Harvested gall-bladder from BDL and control groups were either prepared as whole-tissue slices or microsomal membrane fractions (see below).

Gall-bladder slice preparations

Gall-bladder tissue slices were utilized to examine the synthesis and release of prostanoids from control and BDL groups. The control and BDL groups were prepared under sterile operative conditions as previously described [2–7]. Gall-bladders were then removed from control and BDL groups, cut into slides and placed in oxygenated Krebs–Henseleit buffer, pH 7.4, at 37 °C to measure basal slice release of 6-oxo-PGF_{1α}, a stable metabolite of PGI₂, and PGE₂. The gall-bladder slice was removed after 60 min, and the bath solution was immediately frozen at –20 °C and stored until analysed for prostanoids by radioimmunoassay (r.i.a.). The tissue slices were weighed, then homogenized in 0.1 M-potassium phosphate buffer/0.01 % Triton with a Ultra-Turrax homogenizer (Tekmar, Cincinnati, OH, U.S.A.) for 150 s. The homogenate was clarified by centrifugation (8000 g, 10 min) and the supernatant decanted and analysed for protein content by the method of Bradford [18a].

R.i.a. of arachidonic acid metabolites

Our methodology for radioimmunoassay of 6-oxo-PGF_{1α} and PGE₂ has been described previously [19]. In brief, 6-oxo-PGF_{1α} and PGE₂ were measured from the unextracted bath solution by r.i.a. All samples and standards were measured in duplicate.

Antisera were obtained in freeze-dried form from Dr. Lawrence Levine, Department of Biochemistry, Brandeis University (anti-6-oxo-PGF_{1α} serum) and Advanced Magnetics, Cambridge, MA (anti-PGE₂ serum). Dilutions in assay buffer of 1:5000 and 1:8000 (v/v) for PGE₂ and 6-oxo-PGF_{1α} respectively were used to obtain 40–60 % binding of a zero standard 'blank'. Cross-reactivity of antisera from Dr. L. Levine has been published, and for 6-oxo-PGF_{1α} was < 1 % to PGF_{2α}, PGD₂, thromboxane B₂ and 13,14-dihydro-PGE₂ [20]. Cross-reactivity of the PGE₂ antiserum was given by Advanced Magnetics as 50 % to PGE₁ and < 1 % 6-oxo-PGF_{1α}.

The standard curve was constructed using the smoothed-spline function (r.i.a. computer software package SecuRIA; Packard Instruments, Downers Grove, IL, U.S.A.). Unbound, unabsorbed tracer (referred to as 'non-specific binding') was quantified in tubes containing only labelled ligand and dextran-coated charcoal. Inter-assay and intra-assay variation was 7–9 % for 6-oxo-PGF_{1α} and 14–15 % for PGE. Sensitivity (the amount of unlabelled standard causing 5 % decrease from maximal binding of the blank) was approx. 30 pg/ml. Data are expressed as ng/mg of protein (mean ± S.E.M.).

Preparation of microsomal membrane fractions

The method of preparation of microsomal membrane fractions has been described for several different tissues (our laboratory and others), including kidney, arteries, brain, gastrointestinal tract and gall-bladder [6,21–27]. Harvested gall-bladders from control and BDL groups were weighed and added to 3 vol. (w/v) of oxygenated 100 mM-potassium phosphate buffer with 10 mM-EDTA (to chelate Ca²⁺, thus inhibiting any Ca²⁺-dependent tissue phospholipases) and 1 % fatty-acid-poor bovine BSA (Calbiochem; to absorb any released arachidonic acid), pH 7.4, at 4 °C [22,23]. The tissue was minced, and the resulting suspension was homogenized on ice with a Polytron instrument and spun down at 8000 g for 15 min in a Sorvall centrifuge at 4 °C. The supernatant was removed and centrifuged for 65 min at 105000 g in a refrigerated Beckman L7-55 ultracentrifuge. The surface of the microsomal pellet was carefully washed with

100 mM-potassium phosphate buffer, pH 7.4. The pellet was resuspended in 100 mM-potassium phosphate buffer, pH 7.4, at 4 °C and adjusted to obtain a protein concentration of 0.1 mg/100 μl aliquot to be used in the assay. The reactions took place in silicone-treated (Prosil-28; Baxter Scientific Products, McGaw Park, IL, U.S.A.) 12 mm × 75 mm tubes. Each tube received 100 μl of the microsomal suspension containing 0.1 mg of protein [18a]. The microsomal suspension was incubated with increasing levels of [¹⁴C]arachidonic acid (3 nM–3 μM) in the presence of 1.2 mM-L-adrenaline and 1 mM-GSH at 37 °C for 2 min. Our preliminary experiments identified 750 ng of the labelled [¹⁴C]arachidonic acid to be in excess and not to limit prostanoid synthesis. The 2 min period of incubation was derived from preliminary time-course studies which demonstrated linear prostanoid synthesis up to 20 min of incubation using 0.75 μg of the labelled substrate. When inhibitors were used, 5 mM-imidazole (an inhibitor of thromboxane synthetase) or 10 μM-indomethacin (a cyclo-oxygenase inhibitor) were added to the microsomes [26,27]. Indomethacin, added to the 'in vitro' reaction mixture as a control, decreased total prostanoid release in all normal and BDL groups [29]. Imidazole decreased thromboxane B₂ synthesis in these groups as well [30]. The reaction was terminated by acidification to pH 3.0 with 4 M-formic acid. The reaction mixture was extracted twice with 2 vol. of ethyl acetate. The supernatant was collected, dried under nitrogen and reconstituted with chloroform/methanol (2:1, v/v). The microsomal extracts were subjected to t.l.c. in solvent system A9, the organic phase of ethyl acetate/2,2,4-trimethylpentaneacetic acid/water (10:5:2:10, by vol.) [31]. Authentic unlabelled prostanoid standards were added to the thin-layer plates and identified by the use of I₂ vapour. The radioactive zones corresponding to the prostanoid standards were located, and their radioactivities were counted in liquid-scintillation cocktail (Aquasure; du Pont–NEN, Doraville, GA, U.S.A.) with a LKB 1217 liquid-scintillation counter. Data were calculated as ng (of total or individual PG) per min per mg of protein (mean ± S.E.M.; n = 6 all groups). The substrate levels utilized were plotted against the rate of product formation by the method described by Eisenthal & Cornish-Bowden [14] and Merino [15] to calculate apparent V_{max} (V_{max}) and the apparent K_m (K_m) for individual and total PG synthesis.

Immunoblot analysis

Total microsomal proteins (15 μg) from control and 3DBDL rabbit gall-bladders were separated by 7 %–PAGE in the presence of SDS and transferred on to nitrocellulose filters [16–18]. The filters were blocked for 1 h at room temperature in 100 ml of PBS containing 0.05 % Tween 20, 0.5 M-NaCl and 1 % BSA (Buffer A) and incubated overnight at room temperature in the same solution containing a rabbit a-prostacyclin synthase IgG (1:10000, v/v) (kindly provided by Dr. William L. Smith, Michigan State University, East Lansing, MI, U.S.A.) [32–34]. Filters were washed twice for 20 min in Buffer A at room temperature, then once for 20 min in PBS containing 0.5 M-NaCl and 1 % BSA (buffer B). The blots were incubated for 2 h at room temperature in 100 ml of Buffer B containing 10 μCi of ¹²⁵I-Protein A (sp. radioactivity 1110 MBq/mg) and washed twice for 10 min in Buffer B and finally washed for 10 min in PBS. Immunoreactions were developed by autoradiography overnight at –80 °C (du Pont). Autoradiograms were scanned using the LKB2222-020 Ultrosan XL laser densitometer at 633 nm. Peaks were integrated using the LKB internal integrator and line printer. Replicate scans were within ± 1.00 % and were background-corrected. The mean peak areas and standard errors (mean ± S.E.M.) were calculated for six control and six 3DBDL animals.

RESULTS

Gall-bladder microsomal arachidonic acid metabolism

A comparison of the kinetics of the cyclo-oxygenase activity present in the BDL and control groups were performed by using various amounts of arachidonic acid as substrate under conditions of linear initial velocity. The maximum velocity (V_{max}) for the enzymic conversion of [14 C]arachidonic acid into total PGs synthesized by the control gall-bladder microsomal fractions was 1876 pmol/min per mg of microsomal protein at 37 °C (included 6-oxo-PGF $_{1\alpha}$, PGE $_2$, PGF $_{2\alpha}$, PGA $_2$, PGD $_2$ and thromboxane B $_2$). BDL for 24 and 72 h increased the V_{max} for conversion of [14 C]arachidonic acid into total PG products by gall-bladder microsomal fractions to 2770 pmol/min per mg of microsomal protein and 2663 pmol/min per mg of microsomal protein respectively (Table 1; Fig. 1). BDL for 24 and 72 h did not alter the substrate affinity (K_m).

Table 1. Effect of BDL on rabbit gall-bladder total cyclo-oxygenase activity

Gall-bladder microsomes prepared from the control and 24 h and 72 h BDL groups were incubated with increasing amounts of [14 C]arachidonic acid (3 nM–3 μ M) in the presence of 1.2 mM-L-adrenaline and 1 mM-GSH for 2 min at 37 °C. Extracts were chromatographed as described in the Materials and methods section. In some incubations, microsomes were preincubated with indomethacin before incubation with labelled arachidonic acid, a procedure that completely abolished the response. V_{max} (apparent V_{max}) and K_m (apparent K_m) were calculated by the kinetic analysis described by Eisenthal and Cornish-Bowden [14] and Merino [15]. The values are means with the standard errors ranging from 10 to 30 % ($n = 6$, all groups).

Group	V_{max} . (pmol/min per mg of protein)	K_m (μ M)
Control	1876	1.8
BDL		
24 h	2770	2.1
72 h	2663	2.2

Table 2. Effect of BDL on rabbit gall-bladder microsomal 6-oxo-PGF $_{1\alpha}$ and PGE $_2$ synthesis

Gall-bladder microsomes prepared from the control and 24 h- and 72 h-BDL groups were incubated with increasing amounts of [14 C]arachidonic acid (3 nM–3 μ M) in the presence of 1.2 mM-L-adrenaline and 1 mM-GSH for 2 min at 37 °C. Extracts were chromatographed as described in the Materials and methods section. In some incubations, microsomes were preincubated with indomethacin before incubation with labelled arachidonic acid, a procedure that completely abolished the response. V_{max} (apparent V_{max}) and K_m (apparent K_m) were calculated by the kinetic analysis described by Eisenthal & Cornish-Bowden [14] and Merino [15]. The values are means with the standard errors ranging from 10 to 30 % ($n = 6$ all groups).

Group	6-oxo-PGF $_{1\alpha}$		PGE $_2$	
	V_{max} . (pmol/min per mg of protein)	K_m (μ M)	V_{max} . (pmol/min per mg of protein)	K_m (μ M)
Control	378	1.6	403	1.6
BDL				
24 h	938	1.9	914	1.8
72 h	1049	1.8	928	1.9

Fig. 1. Comparison of Eisenthal & Cornish-Bowden [14] plots of data derived for total PG synthesis from control and 24 h-BDL gall-bladders

Microsomal membrane fractions were prepared as described in the Materials and methods section from control and 24 h-BDL groups. The microsomal suspensions were incubated with increasing levels of [14 C]arachidonic acid (3 nM–3 μ M) in the presence of cofactors for 2 min. The microsomal extracts were subjected to t.l.c. as described in the Materials and methods section. The substrate levels utilized were plotted against the rate of product formation (pmoles prostanoid synthesized/mg protein/minute) by the method described by Eisenthal and Cornish-Bowden and Merino [14,15] to calculate apparent V_{max} (V_{max}) and the apparent K_m (K_m) for total prostanoid synthesis.

The maximal velocities for the enzymic conversion of labelled arachidonic acid to 6-oxo-PGF $_{1\alpha}$ (PGI $_2$ metabolite) and PGE $_2$ are presented in Table 2. The maximal velocity of enzymic conversion of [14 C]arachidonic acid into 6-oxo-PGF $_{1\alpha}$ was 375 pmol/min per mg of microsomal protein, BDL increased the maximal velocity of conversion of labelled arachidonic acid into 6-oxo-PGF $_{1\alpha}$ to 938 pmol/min per mg of microsomal protein in the 24 h-BDL group and to 1049 pmol/min per mg of microsomal protein after 7 h of BDL (Table 2; Fig. 2). The maximal velocity of enzymic conversion of [14 C]arachidonic acid into PGE $_2$ to 403 pmol/min per mg of microsomal protein in the control gall-bladder group. BDL for 24 and 72 h increased the maximal velocity of enzymic conversion of [14 C]arachidonic acid into PGE $_2$ to 914 and 928 pmol/min per mg of microsomal protein respectively (Table 2). BDL did not alter substrate affinity (K_m) for 6-oxo-PGF $_{1\alpha}$ or PGE $_2$ formation (Table 2; Fig. 2).

Table 3. Effect of BDL on rabbit gall-bladder $\text{PGF}_{2\alpha}$, PGD_2 and PGA_2 synthesis

Gall-bladder microsomes prepared from the control and 24 h- and 72 h-BDL groups were incubated with increasing amounts of [^{14}C]arachidonic acid (3 nM–3 μM) in the presence of 1.2 mM-L-adrenaline and 1 mM-GSH for 3 min at 37 °C. Extracts were chromatographed as described in the Materials and methods section. In some incubations, microsomes were preincubated with indomethacin before incubation with labelled arachidonic acid, a procedure that completely abolished the response. V_{max} , (apparent V_{max}) and K_m (apparent K_m) were calculated by the kinetic analysis described by Eisenthal & Cornish-Bowden [14] and Merino [15]. The values are the means, with the standard errors ranging from 10 to 30 % ($n = 6$).

Group	$\text{PGF}_{2\alpha}$		PGD_2		PGA_2	
	V_{max} , (pmol/min per mg of protein)	K_m (μM)	V_{max} , (pmol/min per mg of protein)	K_m (μM)	V_{max} , (pmol/min per mg of protein)	K_m (μM)
Control	282	1.4	255	2.1	269	1.4
BDL						
24 h	349	2.0	242	2.8	309	2.0
72 h	202	1.0	189	3.5	116	2.8

Fig. 2. Comparison of Eisenthal & Cornish-Bowden [14] plots of data which examined synthesis of 6-oxo- $\text{PGF}_{1\alpha}$ derived from control and 24 h BDL gall-bladders

Microsomal membrane factors were prepared as described in the Materials and methods section from control and 24 h-BDL groups. The microsomal suspensions were incubated with increasing levels of [^{14}C]arachidonic acid (3 nM–3 μM) in the presence of cofactors for 2 min. The microsomal extracts were subjected to t.l.c. as described in the Materials and methods section. The substrate levels utilized were plotted against the rate of product formation (pmol of prostanoids synthesized/min per mg of protein) by the method described by Eisenthal & Cornish-Bowden [14] and Merino [15] to calculate apparent V_{max} (V_{max}) and the apparent K_m (K_m) for 6-oxo- $\text{PGF}_{1\alpha}$ synthesis.

Synthesis rates of the other primary prostanoids ($\text{PGF}_{2\alpha}$, PGD_2 and PGA_2) are presented in Table 3. Unlike the data for 6-oxo- $\text{PGF}_{1\alpha}$ and PGE_2 , synthesis of the other primary prostanoids decreased after 3 days of BDL and showed an increased K_m for PGD_2 and PGA_2 after 3 days of BDL (Table 3). Synthesis of thromboxane B_2 in the control and BDL groups was minimal and is not shown in Table 3.

Gall-bladder slice PG synthesis and release

Gall-bladder slice basal synthesis and release of 6-oxo- $\text{PGF}_{1\alpha}$ and PGE_2 are presented in Table 4. Control gall-bladder slice basal 6-oxo- $\text{PGF}_{1\alpha}$ release was 37.4 ± 4.9 ng/mg of protein. BDL increased basal gall-bladder-slice 6-oxo- $\text{PGF}_{1\alpha}$ release to 105.1 ± 24.9 ng/mg of protein after 24 h of BDL and 94.7 ± 23.6 ng/mg of protein after 72 h of BDL. Control gall-bladder-slice basal PGE_2 release was 11.9 ± 4.0 ng/mg of protein. BDL for 24 or 72 h did not alter basal gall-bladder-slice PGE_2 release and was 23.0 ± 8.4 and 17.5 ± 4.4 ng/mg of protein respectively.

Gall-bladder-slice basal release of thromboxane B_2 (a metabolite of thromboxane A_2) was 3-fold less than PGE_2 or 6-oxo- $\text{PGF}_{1\alpha}$ release in the control and experimental groups. There were no significant differences in gall-bladder-slice release of thromboxane B_2 between the control and experimental groups.

Immunoblot analysis

Since BDL increased both the synthesis rate and release of 6-oxo- $\text{PGF}_{1\alpha}$ and PGE_2 , total microsomal membrane protein fractions were prepared from control and BDL gall-bladders and analysed by immunoblot analysis with antibodies against prostacyclin synthase (Fig. 3). The total protein profiles of the microsomal membrane fractions were not significantly different in control and 3DBDL animals (Fig. 3a). However, BDL increased the gall-bladder microsomal content of prostacyclin synthase (Fig. 3b). When probed with a specific antibody against prostacyclin synthase a single immunoreactive protein that migrated with an M_r of 56000 was detected in protein blots after SDS/PAGE. The mean peak areas of the immunoreactive bands was determined densitometrically and was 0.136 ± 0.013 for control animals and 0.672 ± 0.128 for the 72 h-BDL animals. This represents a significant 5-fold increase ($P < 0.002$) in prostacyclin synthase content in the gall-bladder microsomes obtained from the 72 h-BDL group compared with the control (Fig. 3b).

Table 4. Effect of BDL on rabbit gall-bladder tissue slice PG release

Gall-bladder slices were incubated in 5 ml of oxygenated Krebs-Henseleit buffer, pH 7.4, at 37 °C for 60 min. Unextracted buffer was removed and 6-oxo-PGF_{1α} and PGE₂ were measured by r.i.a. as described in the Materials and methods section. Results are presented as ng of PG/mg of protein (mean ± S.E.M.). *indicates significance at *P* < 0.02 compared with control, and **indicates significance at *P* < 0.04 compared with control level (Students' two-tailed *t* test).

Group	<i>n</i>	Release (ng of PG/mg)	
		6-oxo-PGF _{1α}	PGE ₂
Control	8	37.4 ± 4.9	11.9 ± 4.0
BDL			
24 h	9	105.1 ± 11.8*	23.0 ± 8.4
72 h	9	94.3 ± 23.6**	17.5 ± 4.4

DISCUSSION

BDL in the rabbit was described recently as a new animal model of acute cholecystitis [2–7]. BDL for 6, 24 and 72 h induced both gall-bladder microsomal cyclo-oxygenase activity and histological changes identical with that observed in human early acute cholecystitis [6]. The major gall-bladder microsomal prostanoids synthesized after all time periods of BDL were PGI₂ and PGE₂. Previously we showed that PGI₂ inhibits gall-bladder water transport *in vitro* in a dose responsive manner. Thus elevated levels of PGI₂ in the gall-bladder likely play a central role in the biochemical and physiological changes associated with acute gall-bladder inflammation [8]. Two other laboratories reported similar findings in two different animal models of acute cholecystitis [35,36]. The present study, however, provides a possible mechanism for the increased gall-bladder microsomal synthesis of prostacyclin after BDL.

Two different biochemical measures were utilized to determine the mechanism of increased gall-bladder microsomal prostanoid synthesis after BDL. A kinetic enzyme analysis [14–15] and the technique of protein blot analysis [16–18] were used to define the specific arachidonic acid metabolic pathway that leads to increased prostaglandin content in the inflamed gall-bladder [14,15]. BDL stimulated a quantitative change in the enzymes involved with gall-bladder conversion of arachidonic acid into 6-oxo-PGF_{1α} and PGE₂. Gall-bladder microsomal membrane fractions when incubated with increasing levels of [¹⁴C]-arachidonic acid had a significant 3-fold increased rate of conversion (*V*_{max}) into PGI₂ and PGE₂ and no changes in the apparent substrate affinity (*K*_m). Conversely, kinetic analyses for the conversion of [¹⁴C]arachidonic acid into three other prostaglandins, PGF_{2α}, PGD₂ and PGA₂, showed decreases in the apparent *V*_{max} and increases in the apparent *K*_m of each of these respective reactions. When the rate for total PG synthesis after BDL for 24 and 72 h was calculated, *V*_{max} was increased 47% and 41% respectively when compared with control groups (Table 1).

Since quantitative changes in the synthesis rates of specific arachidonic acid metabolites were observed, the specific content of the enzymes responsible for the synthesis of 6-oxo-PGF_{1α} and PGE₂ were measured by protein blot and immunoreaction with antibodies against cyclo-oxygenase and prostacyclin synthase. The total protein profiles of the control and inflamed gall-bladder microsomes were unchanged by inflammation. Acute inflammation induced only a slight increase in cyclo-oxygenase content (results not shown), but a 5-fold increase in prostacyclin synthase content was measured in the microsomes from 72 h-BDL as compared with controls. The data from these two independent methods are consistent with the presence of more total enzymes responsible for 6-oxo-PGF_{1α} and PGE₂ synthesis rather than a change in enzyme affinity or the appearance of a novel enzyme.

BDL for 72 h increased the *V*_{max} for total PG synthesis by 41% and increased the *V*_{max} for 6-oxo-PGF_{1α} and PGE₂ by 3-fold. This discrepancy was explained by the decreased *V*_{max} values for PGF_{2α}, PGD₂ and PGA₂ and the increased *K*_m or change in affinity of the specific enzymes responsible for conversion of PGH₂ into PGD₂ and PGA₂. These data support our previous study, which examined sex differences during evolving gall-bladder inflammation. Total PG synthesis was 4-fold higher in the control female rabbit gall-bladder microsomes than the male. BDL for 3 days only moderately increased total PG synthesis in the female rabbit gall-bladder, dramatically increased gall-bladder synthesis of 6-oxo-PGF_{1α} and decreased synthesis of all other primary prostanoids. Unlike the female rabbit gall-bladder, the male rabbit gall-bladder significantly increased total

Fig. 3. Immunoblot analysis of gall-bladder microsomes for prostacyclin synthase content

The results of SDS/7%-PAGE of total solubilized microsomal proteins (25 µg/lane are shown). Lanes 1–6 are controls; lanes 7–12 are 72 h BDL. (a) Coomassie Blue Stain; (b) a-Prostacyclin synthase IgG experiments were performed in triplicate with three independent groups of animals (six animals per condition). MS, *M*_r standards. Arrows denote the position of the immunoreactive protein. Peak areas were determined by using an LKB 2222-020 UltraScan XL laser densitometer. Means ± S.E.M. were calculated. A significant (*P* < 0.002; Student's two-tailed *t* test) increase in specific prostacyclin synthase content was observed in the 72 h-BDL animals.

PG synthesis after BDL, with the major PG synthesized being 6-oxo-PGF_{1α} [37].

Previous studies examining the effects of pathological stimuli on whole-organ or cellular cyclo-oxygenase activity have shown similar results [22–24,38–43]. Several of these studies demonstrated that increased prostanoid synthesis after pathological stimuli was due to new protein and RNA synthesis [22,39]. The present data also suggested that a similar mechanism involving new protein synthesis increases the total gall-bladder cyclo-oxygenase activity after BDL.

A comparison of control and BDL gall-bladder-slice basal PGI₂ and PGE₂ synthesis and release is presented in Table 4. Control gall-bladder release of PGI₂ was 3-fold higher than that of PGE₂. BDL for 24 and 72 h significantly increased gall-bladder basal 6-oxo-PGF_{1α} release, but not that of PGE₂, when compared with the control. BDL-stimulated 6-oxo-PGF_{1α} release was 3- and 5-fold higher than BDL-stimulated PGE₂ release after 24 and 72 h of BDL respectively. Similar to the situation with BDL groups, control gall-bladder-slice 6-oxo-PGF_{1α} release was 3-fold higher than control gall-bladder-slice PGE₂ release. Although BDL similarly stimulated the rate of gall-bladder microsomal substrate conversion (V_{max}) into both PGE₂ and 6-oxo-PGF_{1α}, whole-gall-bladder tissue showed a preferential release for 6-oxo-PGF_{1α} over PGE₂ release.

Prostanoids are paracrine substances that are produced by the entire gastrointestinal tract, including the gall-bladder [6,21,25]. Previous studies *in vitro* have shown that PGI₂ decreased gall-bladder normal fluid absorption when applied exogenously to rabbit gall-bladder [8]. We hypothesized that increased gall-bladder PGI₂ synthesis is an important factor contributing to gall-bladder distension and mucosal disruption, two important stages in the early onset of acute cholecystitis. Our findings of increased synthesis and release of gall-bladder PGI₂ after rabbit BDL support this hypothesis. BDL in the rabbit stimulated increased preferential endogenous PGI₂ synthesis and release, as indicated by our microsomal kinetic and tissue-slice experiments. A possible model is presented in which the net result of increased gall-bladder PGI₂ is decreased fluid absorption, causing increased gall-bladder distension, subsequent mucosal injury and the onset of clinical acute cholecystitis.

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