Effects of endotoxin and dexamethasone on group I and II phospholipase A₂ in rat ileum and stomach

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Abstract

Phospholipase A₂ (EC 3.1.1.4) is a key enzyme in inflammation and is thought to play an important part in inflammatory diseases of the gastrointestinal tract. To investigate the nature and regulation of phospholipase A₂ activity in the gastrointestinal mucosa, the distribution of messenger ribonucleic acid (mRNA) for group II phospholipase A₂ in various parts of the rat gastrointestinal tract was studied, as well as the influence of endotoxin or dexamethasone, or both, on the group I and II phospholipase A₂ mRNA expression and activity in the rat glandular stomach and distal ileum. The results show that (a) group II phospholipase A₂ is present along the whole gastrointestinal tract, but in particularly large amounts in the distal ileum, (b) endotoxin increases group II, but not group I, phospholipase A₂ mRNA expression in the glandular stomach and distal ileum, and (c) dexamethasone reduces the endotoxin induced increases in group II phospholipase mRNA expression and activity in the gastrointestinal mucosa. These findings suggest that phospholipase A₂ of type II is a mediator of endotoxin effects in the gastrointestinal mucosa and that its expression at the mRNA level can be inhibited by corticosteroids.

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Phospholipase A₂ (PLA₂) is a key enzyme in the formation of arachidonic acid metabolites, platelet activating factor, and lysophosphatidylcholine, all of which have been implicated in inflammatory reactions in the gastrointestinal tract.¹ For example, increased values of arachidonic acid metabolites^{2,3} and platelet activating factor⁴ have been shown in the intestinal mucosa of patients with Crohn's disease, suggesting that intestinal PLA₂ activation may play a part in the pathogenesis of this disorder. Moreover, in Crohn's disease, increase in mucosal PLA₂ activity seems to precede the intestinal inflammation.^{5,6} Detailed characterisation of the intestinal PLA₂ activity, however, is as yet lacking and the precise mechanisms responsible for the increased intestinal PLA₂ activity in Crohn's disease remain to be identified.

The phospholipases A₂ of the gastrointestinal tract⁷⁻¹¹ include both high molecular weight^{10,12} and low molecular weight⁷⁻⁹ isoforms, the low molecular phospholipases A₂ being divided into group I and group II types.¹³ Phospholipases A₂ from group I are found in large amounts in the pancreas⁷ and are thus thought to serve mainly as digestive enzymes.¹¹ The presence of group I PLA₂, however, in organs other than the pancreas, such as the lung⁷ and spleen,¹⁴ suggests

that group I phospholipases A₂ have additional, yet unidentified, physiological functions. The rat glandular stomach contains high concentrations of messenger ribonucleic acid (mRNA) for group I PLA₂,^{7,8} whereas other parts of the rat gastrointestinal tract seem to lack group I PLA₂.⁸ Group II PLA₂ mRNA, on the other hand, is more widely distributed in the rat gastrointestinal tract,⁹ with particularly large amounts found in the ileum.⁹ As group II phospholipases A₂ are thought to be important in inflammatory reactions¹⁵⁻¹⁷ and as the ileum is the predilection site of Crohn's disease,¹⁸ the question arises as to how the small intestinal group II PLA₂ activity is regulated, and how it may be affected by inflammatory promoting agents.

It has previously been shown that endotoxin and dexamethasone may affect the mRNA expression of group II PLA₂ in the lung and spleen of endotoxin shock rats.¹⁹ In this study, we have investigated the effects of endotoxin and dexamethasone on the group II PLA₂ mRNA expression in the rat ileum, and, for comparison, on the group I and II PLA₂ mRNA expression in the rat glandular stomach. Our results suggest that endotoxin may increase the intestinal group II PLA₂ activity by increasing the activity of group II PLA₂ mRNA, and that this increase is reduced by dexamethasone. Moreover, the results show that group II PLA₂ and group I PLA₂ are regulated differently in the rat glandular stomach, as only the group II PLA₂ mRNA expression (but not the group I PLA₂ mRNA expression) was affected by endotoxin and dexamethasone.

Material and methods

CHEMICALS

Endotoxin (lipopolysaccharide from *Escherichia coli*, 055:B5), dexamethasone, and *Naja naja* venom PLA₂ were purchased from Sigma, St Louis, Mo, USA. *Naja naja* venom PLA₂ contained about 70% protein and with an activity of 1300 units per mg protein; one unit hydrolyses 1.0 µmol of soybean L-α-phosphatidylcholine to L-α-lysophosphatidylcholine and fatty acid per minute at pH 8.9 at 25°C.

PROBES

For detection of group II PLA₂, an oligonucleotide (obtained from Operon Technologies, Alameda, USA), corresponding to base pairs 701-435 in the rat membrane associated phospholipase A₂ complementary deoxyribonucleic acid, sequenced from rat spleen by Ishizaki *et al*,⁹ was used. The sequence of the probe was 5'-GCTTTTCTTGTTCCGGGCAA

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AACATTTCAGCGGCAG. This sequence is specific for rat group II PLA₂ as ascertained by computer assisted search of updated versions of Gene Bank.

For detection of group I PLA₂, a deoxyribonucleic acid sequence corresponding to the coding region of rat group I PLA₂, cloned and sequenced from rat pancreatic tissue by Ohara *et al.*²⁰ was used.

The oligonucleotide probe for PLA₂-II was 5'-end labelled (5'-End Labeling Kit, Boehringer Mannheim, Mannheim, Germany) with adenosine 5'-[γ-³²P]-triphosphate (5000 Ci/mmol; Amersham, UK). The complementary deoxyribonucleic acid probe for PLA₂-I was labelled with deoxycytidine 5'-[α-³²P]-triphosphate (3000 Ci/mmol; Amersham, UK) by random priming (Random Prime Kit, Boehringer Mannheim, Mannheim, Germany).

ANIMALS

Male Sprague-Dawley rats (ALAB, Stockholm, Sweden) weighing 230–300 g were used. The study was approved by the Ethics Committee for Animal Experimentation, Linköping. The animals had free access to standard food pellets and tap water throughout the experiments. Within the groups, treated animals were matched to controls with regard to body weight. Before the endotoxin was given, the rats were anaesthetised with ketamine hydrochloride (Ketalar, Parke-Davis, UK) and xylazine chloride (Rompun, Bayer, Germany). The animals were killed by cervical dislocation.

EXPERIMENTAL DESIGN

In the first experiment nine rats were injected intravenously with endotoxin (5 mg/kg body weight, dissolved in saline), and nine rats were injected with an equal volume of saline. At different time points after injection (6, 12, and 24 hours later), three rats from each group were killed. The glandular stomach and 10 cm of the distal ileum were immediately taken out and washed in ice cold saline before being freeze-dried in liquid nitrogen and kept at –70°C until analysed.

In a second experiment, 32 rats were divided into four groups with eight rats in each. One group was injected intravenously with endotoxin (5 mg/kg body weight, dissolved in saline), and one group received an intraperitoneal injection of dexamethasone (10 mg/kg body weight, dissolved in saline) 30 minutes before the injection of endotoxin. The control groups received either an equal volume of saline intravenously, or dexamethasone intraperitoneally. The rats were killed 12 hours after endotoxin or saline injection. The glandular stomach and 10 cm of the distal ileum were immediately taken out and washed in ice cold saline before being freeze-dried in liquid nitrogen and kept at –70°C until analysed.

TOTAL RNA PREPARATION

Total ribonucleic acid (RNA) was prepared according to Chomczynski and Sacchi.²¹ Briefly,

the tissues were homogenised (100 mg/ml) for 30–45 seconds with a Polytron homogeniser (Brinkmann Instruments, Westbury, NY, USA) in a solution containing 4 mol/l acid guanidinium thiocyanate, 25 mmol/l sodium citrate, pH 7.0, 0.5% sarcosyl, and 0.1 mol/l 2-mercaptoethanol. For extraction of RNA, 0.1 volumes of 2 mol/l sodium acetate, pH 4.0, 1 volume phenol (saturated in water), and 0.2 volumes chloroform-isoamyl alcohol mixture (49:1) were added. After centrifugation at 10 000 g for 20 minutes, the RNA in the aqueous phase was collected, precipitated, and recentrifuged. The RNA pellet was then dissolved in an aliquot of the same solution as used for homogenisation, reprecipitated with 1 volume isopropanol, and centrifuged. The pellet was washed in 70% ethanol, centrifuged, dried, and dissolved in 0.1% lauryl sulphate and kept at –70°C until analysed.

NORTHERN BLOTS

Separation of different sized RNA fragments was performed in a denaturing system: 1% agarose gel in 20 mmol/l 3-[N-morpholino]propanesulphonic acid buffer, pH 7.0, and 2.2 mol/l formaldehyde. Twenty mmol/l 3-[N-morpholino]propanesulphonic acid buffer, pH 7.0, was used as electrophoresis running buffer. Before application to the submarine gel, the RNA was diluted in 20 µl denaturing sample buffer (20 mmol/l 3-[N-morpholino]propanesulphonic acid buffer, pH 7.0, 50% formamide, and 2.2 mol/l formaldehyde), heated to 60°C for five minutes, and mixed with 2 µl of a solution containing 0.4% bromophenolblue and 20% Ficoll. The amount of total RNA in each application slit for northern blot was 30 µg for stomach and 10 µg for distal ileum. After electrophoretic separation of the RNA fragments, the gel was soaked in denaturing buffer (0.05 mol/l sodium hydroxide and 0.15 mol/l sodium chloride) for 30 minutes. RNA was then transferred to a Zeta-Probe GT cationised nylon membrane (Bio-Rad Laboratories, Brussels, Belgium). The transfer was performed under pressure for 15 minutes, by the use of a PosiBlot pressure blotter (Stratagene, La Jolla, USA). Because the transfer was performed in an alkaline solution (0.05 mol/l sodium hydroxide), no further fixation of RNA to the membrane was done. The membrane was gently washed in a saline-sodium citrate buffer (0.39 mol/l sodium chloride and 30 mmol/l sodium citrate, pH 7.0) and sealed in a plastic bag until hybridisation. The PLA₂-II hybridisation reaction was carried out in a solution containing 0.39 mol/l sodium chloride and 0.03 mol/l sodium citrate, 5×Denhardt's solution (Denhardt's solution is 0.02% each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll), 2% lauryl sulfate, 100 µg/ml denatured, fragmented salmon sperm DNA, and ³²P-labelled PLA₂-II probe (15×10⁶ dpm/l). The membrane and the solution were sealed in a plastic bag, and slowly moved in a waterbath (62°C) for three hours. The membranes were washed and the resulting blots were subjected to autoradiography on Cronex 4 x ray film, in cassettes with intensifying screens (Du Pont de Nemours, Bad Homburg, Germany) at –70°C

before development. After stripping in 0.1% lauryl sulphate at 95°C for 10 minutes, a second hybridisation, for detection of group I PLA₂, was performed. The conditions for hybridisation with the complementary deoxyribonucleic acid sequence have been described previously.⁸

RNA DOT BLOTS

RNA preparations were transferred directly to Zeta-Probe GT, cationised nylon membrane (Bio-Rad Laboratories, Brussels, Belgium) with a microfiltration unit according to the manufacturers instruction (Bio-Rad). RNA was precipitated and diluted in ice cold 10 mmol/l sodium hydroxide and 1 mmol/l EDTA to give a final amount of 2 µg total RNA for ileum, (8 µg for glandular stomach). After the application of RNA, the membrane was hybridised and autoradiographed according to the procedure described for northern blots, but with the labelled probe in a concentration of 1.5×10⁹ dpm/l hybridisation solution. The signal intensity was measured with a computerised image system (Bio Image Products, Ann Arbor, MI, USA). Suitable spots for detecting PLA₂-II in ileum were seen after nine hours of exposure (120 hours for glandular stomach). The mean values from measurements of two spots were used in the calculations.

PLA₂ ENZYME ACTIVITY

PLA₂ activity was analysed in freeze dried specimens from the glandular stomach and ileum. The tissue was pulverised before being weighed (1 g dry weight/l saline) and homogenised with a Polytron disintegrator 3×10 seconds. The enzyme activity was analysed as previously described.²² In brief, ¹⁴C-oleic acid labelled *E coli* (15 000 cpm) was used as substrate in a reaction mixture buffered to pH 7.4 and containing 2.5 mmol/l calcium chloride. The sample homogenate was diluted to give a final amount of 0.5 µg (dry weight) tissue in the assay. The reaction mixture was incubated at 37°C for one hour and the fatty acid released was measured after filtration through a 0.45 µm filter. The calculated enzyme activities of the samples were based on linear regression analysis *v Naja naja* venom PLA₂ standard. Five activity points, in the range of 3 to 3×10³ units/l, were analysed in duplicate and in parallel with the tissue samples. After background correction, ¹⁴C-oleic acid liberation of total radioactivity, was plotted on the x axis and the *Naja naja* venom PLA₂ concentration, expressed as U/l (natural logarithm) on the y axis. The straight line was fitted by the least square method and gave a correlation coefficient *r* of 0.997. The mean values from two measurements of the samples are shown.

STATISTICAL ANALYSIS

Comparative statistics of the different groups included in the study was made with two tailed Mann-Whitney U rank sum test, with *p*<0.05 considered significant. In the test of correlation, Spearman's correlation coefficient was used and *p*<0.05 considered significant.

Results

GROUP II PLA₂ mRNA EXPRESSION IN THE RAT GASTROINTESTINAL TRACT

Group II PLA₂ mRNA was detected in all tissues examined – that is, the forestomach, glandular stomach, duodenum, ileum, caecum, and colon. About the same amounts were found in the stomach, duodenum, and colon. The largest amounts were found in the distal part of ileum (Fig 1).

EFFECT OF ENDOTOXIN ON THE PLA₂ mRNA EXPRESSION

In both the glandular stomach and the distal part of ileum, the type II PLA₂ mRNA expression was increased six hours after endotoxin injection, and did not seem to decline during the next 18 hours (Table). In contrast, the glandular stomach PLA₂ type I mRNA expression was not increased after endotoxin treatment (Table). Type I PLA₂ mRNA was not detected in the ileum, either before or after endotoxin treatment (data not shown).

EFFECT OF DEXAMETHASONE ON THE PLA₂ mRNA EXPRESSION

Twelve hours after the intravenous injection of endotoxin, the expression of PLA₂ type II mRNA was significantly increased in the glandular stomach and the distal ileum (Figs 2 and 3), confirming the above results (Table). When dexamethasone was given intraperitoneally 30 minutes before the endotoxin challenge, however, the increase in PLA₂ type II mRNA expression after 12 hours was reduced both in the glandular stomach (Fig 2) and in the ileum

Figure 1: Northern blot analysis of group II PLA₂ mRNA (corresponding to 0.8 kb), in the rat gastrointestinal tract. Lane a, forestomach; b, glandular stomach; c, duodenum; d, ileum; e, distal ileum; f, caecum; g, proximal colon; h, distal colon. Each lane was loaded with 30 µg total RNA and the nylon filter hybridised with a rat group II PLA₂ oligonucleotide probe.

PLA₂ mRNA expression after endotoxin challenge*

		PLA ₂ mRNA (AU)			
		Glandular stomach		Ileum	
	Time (h)	Control	LPS	Control	LPS
Group I	6	2.6 (0.8)	2.1 (0.6)	ND†	ND
	12	1.9 (2.3)	0.9 (0.2)	ND	ND
	24	3.4 (0.5)	1.3 (0.6)†	ND	ND†
Group II	6	11.8 (1.3)	18.1 (1.0)	5.3 (0.9)	10.4 (0.7)
	12	7.5 (4.1)	15.5 (0.9)	3.0 (2.2)	8.7 (1.2)
	24	15.9 (2.3)	17.4 (3.6)†	6.1 (2.9)	11.4 (0.3)†

*Rats were injected with lipopolysaccharide from *E coli* (LPS) or with saline (control) and killed 6, 12, or 24 hours later. Eight µg total RNA from glandular stomach and two µg from ileum was analysed with Dot blot technique. The results are expressed as densitometric arbitrary units (AU). Mean values (SD) (n=3). †=(n=2). ‡=not detected (ND).

Figure 2: Group II PLA₂ mRNA expression in the rat glandular stomach 12 hours after challenge with lipopolysaccharide from E coli (LPS) and in animals given dexamethasone 30 minutes before the endotoxin challenge (Dex/LPS). Saline alone (saline) and dexamethasone alone (Dex), respectively, was injected in control groups. Eight µg total RNA was analysed with Dot blot technique. The results are expressed as densitometric arbitrary units (AU). Each point represents the value from one animal (eight in each group).

Fig 3). Dexamethasone did not significantly reduce the basal value of PLA₂ type II mRNA in the stomach (Fig 2) or ileum (Fig 3). Endotoxin did not affect the glandular stomach type I phospholipase A₂ mRNA expression, and dexamethasone was without effect (Fig 4).

EFFECT OF ENDOTOXIN AND DEXAMETHASONE ON THE PLA₂ ACTIVITY
The ileal PLA₂ activity was significantly increased 12 hours after intravenous injection of endotoxin (Fig 5). Dexamethasone treatment 30 minutes before endotoxin challenge significantly reduced this increase (Fig 5). The glandular stomach PLA₂ activity, on the other hand, was not affected by endotoxin or dexamethasone (Fig 6).

CORRELATION ANALYSIS OF PLA₂ ENZYME ACTIVITY AND GROUP II PLA₂ mRNA EXPRESSION IN ILEUM
Spearman correlation analyses based on all groups – that is, including 32 animals, showed

Figure 3: Group II PLA₂ mRNA expression in the rat distal ileum 12 hours after challenge with lipopolysaccharide from E coli (LPS) and in animals given dexamethasone 30 minutes before the endotoxin challenge (Dex/LPS). Saline alone (Saline) and dexamethasone alone (Dex), respectively, was injected in control groups. Two µg total RNA was analysed with Dot blot technique. The results are expressed as densitometric arbitrary units (AU). Each point represents the value from one animal (eight in each group).

Figure 4: Group I PLA₂ mRNA expression in the rat glandular stomach 12 hours after challenge with lipopolysaccharide from E coli (LPS) and in animals given dexamethasone 30 minutes before the endotoxin challenge (Dex/LPS). Saline alone (Saline) and dexamethasone alone (Dex), respectively, was injected in control groups. Eight µg total RNA was analysed with Dot blot technique. The results are expressed as densitometric arbitrary units (AU). Each point represents the value from one animal (eight in each group).

good correlation in ileal PLA₂ enzyme activity v group II PLA₂ mRNA expression (coefficient, $r=0.73$; $p<0.0001$) (Fig 7).

Discussion
We have previously found that an increase in mucosal PLA₂ activity precedes the recurrence of intestinal inflammation in Crohn's disease.^{5,6} This finding suggests the possibility that a group II PLA₂ might play a part in Crohn's disease, because group II phospholipases A₂ seem to participate in other inflammatory conditions^{23,24}

Figure 5: PLA₂ enzyme activity in the rat distal ileum 12 hours after challenge with lipopolysaccharide from E coli (LPS) and in animals given dexamethasone 30 minutes before the lipopolysaccharide challenge (Dex/LPS). Controls were given saline alone (Saline) and dexamethasone alone (Dex), respectively. Each point represents the value from one animal (eight in each group).

Figure 6: PLA₂ enzyme activity in the rat glandular stomach 12 hours after challenge with lipopolysaccharide from E coli (LPS) and in animals given dexamethasone 30 minutes before the lipopolysaccharide challenge (Dex/LPS). Controls were given saline alone (Saline) and dexamethasone alone (Dex), respectively. Each point represents the value from one animal (eight in each group).

and also to be stimulated by known inflammatory agents.^{15 19 25-27} Classic Crohn's disease is located in the distal ileum, and after resectional surgery with ileocolonic anastomosis the recurrence will always affect the neoterminal ileum.⁶ These considerations prompted the investigation in this study of the PLA₂ in this part of the gastrointestinal tract.

The investigation showed that the distal ileum is the part of the gastrointestinal tract richest in group II PLA₂ mRNA in rats, and that the ileal expression of group II PLA₂ mRNA is increased in animals given endotoxin. Endotoxin is a known proinflammatory agent^{28 29} and has previously been shown to increase the group II PLA₂ mRNA expression in different cell systems^{15 25} and also in the aorta, spleen, lung, and thymus of endotoxin shock rats.¹⁹ Moreover, only the group II PLA₂ mRNA expression, but not the group I PLA₂ mRNA expression, was increased in the glandular stomach after endotoxin, suggesting that the expression of group II and group I PLA₂ mRNA is regulated differently in this tissue.

It has previously been shown that endotoxin stimulates intestinal mucosal PLA₂ activity in rats as soon as three minutes after an intravenous injection,³⁰ suggesting that endotoxin may stimulate intestinal PLA₂ activity also without

previous induction of mRNA synthesis. It seems probable, therefore, that endotoxin induces intestinal PLA₂ activity by stimulating both post-transcriptional and pre-transcriptional events. Although the precise mechanisms, however, participating in the endotoxin stimulated PLA₂-II activation remain to be investigated, one may speculate that endotoxin exerted its effect in this study by releasing cytokines.³¹ For example, the cytokines tumour necrosis factor and interleukin-1 have both been shown to stimulate PLA₂ activity^{15 25 32-36} and PLA₂-II mRNA expression^{15 25 37} in different cell types.

This study also showed that endotoxin increases the PLA₂ activity of the distal ileum. This is in agreement with previous investigations, showing that endotoxin may increase not only the PLA₂-II mRNA expression, but also the PLA₂-II activity.^{15 25} Our results thus suggest that endotoxin may stimulate ileal group II PLA₂ activity by increasing the gene expression for this enzyme. Although the distal ileum is known to contain more than one type of PLA₂,^{10 38} a correlation analysis based on all groups – that is, including 32 animals, shows a very strong correlation between the ileal group II PLA₂ mRNA activity and the PLA₂ activity (Fig 7). Thus, animals with a high ileal group II PLA₂ mRNA activity also had a high ileal PLA₂ activity. One has to assume, therefore, that the measured ileal PLA₂ activity mainly originated from group II PLA₂. The PLA₂ activity of the glandular mucosa, on the other hand, was not affected by endotoxin despite the increased PLA₂-II mRNA activity. One explanation might be a high level of group I PLA₂ activity in the glandular stomach,^{8 14} as the group I PLA₂ mRNA expression was not affected by endotoxin. Moreover, the basal PLA₂ activity was about 10 times higher in the stomach than in the ileum, suggesting that minor changes of the PLA₂-II activity in the stomach were masked by the high PLA₂-I activity.

Pretreatment with the anti-inflammatory glucocorticoid, dexamethasone, reduced the endotoxin stimulated increase in group II PLA₂ mRNA, both in the ileum and the stomach, whereas the basal activity of group II PLA₂ mRNA, and the group I PLA₂ mRNA of the stomach, were unaffected. Moreover, dexamethasone reduced the PLA₂ activity of the ileum. These findings suggest that dexamethasone specifically inhibited the endotoxin induced increases in group II PLA₂ mRNA and activity. This is in agreement with previous investigations, showing that dexamethasone inhibits group II PLA₂ expression and activity in the aorta, spleen, lung, and thymus of endotoxin shock rats¹⁹ and endotoxin treated cultured rat astrocytes.²⁵ Although the precise mechanism by which dexamethasone inhibited the effect of endotoxin in this study remains to be clarified, one possibility is that dexamethasone induced the synthesis of PLA₂ inhibitory proteins – that is, lipocortins.³⁹ Moreover, as dexamethasone has been shown to suppress endotoxin stimulated transcription and translation of the tumour necrosis factor gene,⁴⁰ one may speculate that dexamethasone inhibited the endotoxin induced increase in PLA₂-II mRNA by reducing the level

Figure 7: Scatter diagram illustrating the correlation between group II PLA₂ mRNA expression (x axis) and PLA₂ enzyme activity (y axis) in rat distal ileum. Correlation coefficient (including 32 animals), $r = 0.73$; $p < 0.0001$. Data from Figures 3 and 5. Challenge with lipopolysaccharide from E coli (n=8) ●, dexamethasone treatment 30 minutes before lipopolysaccharide challenge (n=8) ▲, controls given saline alone (n=8) ○, and controls given dexamethasone alone (n=8) △.

of tumour necrosis factor,²⁹ a known stimulator of PLA₂-II gene expression.^{15 25 37 41}

In summary, we have shown that the distal ileum is the part of the gastrointestinal tract with the most group II PLA₂ mRNA, and that the expression of this mRNA, and the corresponding enzyme activity, may be affected by endotoxin and dexamethasone. We have also shown that the group II PLA₂ activity and the group I PLA₂ activity are regulated differently in the glandular stomach. As far as we know, this is the first study in which the regulation of group I and group II PLA₂ mRNA expression has been investigated in the same organ within the same study. This study does not make clear, however, which particular cell type(s) is responsible for the stomach and ileal PLA₂ activity and PLA₂ mRNA expression. This problem could be considered by studying the gastrointestinal PLA₂ mRNA expression after cell fractionation to yield homogenous cell populations or by in situ hybridisation. Moreover, to clarify the role of phospholipase A₂ in Crohn's disease, further studies are needed to elucidate the distribution and role of group I and II PLA₂ in the normal and diseased human gastrointestinal tract.

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