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Effects of endotoxin and dexamethasone on group I and II phospholipase A_2 in rat ileum and stomach

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Abstract

Phospholipase A_2 (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 A_2 activity in the gastrointestinal mucosa, the distribution of messenger ribonucleic acid (mRNA) for group II phospholipase A_2 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 A_2 mRNA expression and activity in the rat glandular stomach and distal ileum. The results show that (a) group II phospholipase A_2 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 A_2 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 A_2 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 A_2 (PLA_2) 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 factor[4] have been shown in the intestinal mucosa of patients with Crohn's disease, suggesting that intestinal PLA_2 activation may play a part in the pathogenesis of this disorder. Moreover, in Crohn's disease, increase in mucosal PLA_2 activity seems to precede the intestinal inflammation.[5 6] Detailed characterisation of the intestinal PLA_2 activity, however, is as yet lacking and the precise mechanisms responsible for the increased intestinal PLA_2 activity in Crohn's disease remain to be identified.

The phospholipases A_2 of the gastrointestinal tract[7–11] include both high molecular weight[10 12] and low molecular weight[7–9] isoforms, the low molecular phospholipases A_2 being divided into group I and group II types.[13] Phospholipases A_2 from group I are found in large amounts in the pancreas[7] and are thus thought to serve mainly as digestive enzymes.[11] The presence of group I PLA_2, however, in organs other than the pancreas, such as the lung[7] and spleen,[14] suggests that group I phospholipases A_2 have additional, yet unidentified, physiological functions. The rat glandular stomach contains high concentrations of messenger ribonucleic acid (mRNA) for group I PLA_2,[7 8] whereas other parts of the rat gastrointestinal tract seem to lack group I PLA_2.[8] Group II PLA_2 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 A_2 are thought to be important in inflammatory reactions[15–17] and as the ileum is the predilection site of Crohn's disease,[18] the question arises as to how the small intestinal group II PLA_2 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 PLA_2 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 PLA_2 mRNA expression in the rat ileum, and, for comparison, on the group I and II PLA_2 mRNA expression in the rat glandular stomach. Our results suggest that endotoxin may increase the intestinal group II PLA_2 activity by increasing the activity of group II PLA_2 mRNA, and that this increase is reduced by dexamethasone. Moreover, the results show that group II PLA_2 and group I PLA_2 are regulated differently in the rat glandular stomach, as only the group II PLA_2 mRNA expression (but not the group I PLA_2 mRNA expression) was affected by endotoxin and dexamethasone.

Material and methods

CHEMICALS

Endotoxin (lipopolysaccharide from *Escherichia coli*, 055:B5), dexamethasone, and *Naja naja* venom PLA_2 were purchased from Sigma, St Louis, Mo, USA. *Naja naja* venom PLA_2 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 PLA_2, an oligonucleotide (obtained from Operon Technologies, Almeda, USA), corresponding to base pairs 701–435 in the rat membrane associated phospholipase A_2 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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AACATTCAGCGGCAG. This sequence is specific for rat group II PLA_2 as ascertained by computer assisted search of updated versions of Gene Bank.

For detection of group I PLA_2, a deoxyribonucleic acid sequence corresponding to the coding region of rat group I PLA_2, cloned and sequenced from rat pancreatic tissue by Ohara *et al*[20] was used.

The oligonucleotid probe for PLA_2-II was 5′-end labelled (5′-End Labeling Kit, Boehringer Mannheim, Mannheim, Germany) with adenosine 5′-[γ-P^{32}]-triphosphate (5000 Ci/mmol; Amersham, UK). The complementary deoxyribonucleic acid probe for PLA_2-I was labelled with deoxycytidine 5′-[α-^{32}P]-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 anaesthesised with ketamine hydrochloride (Ketalar, Parke-Davis, UK) and xylazin 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 freezed 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 freezed 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% bromphenolblue 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 PLA_2-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 P^{32}-labelled PLA_2-II probe (15×10^9 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 PLA_2, 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 $1 \cdot 5 \times 10^9$ 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 PLA_2-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.

PLA_2 ENZYME ACTIVITY

PLA_2 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, ^{14}C-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 PLA_2 standard. Five activity points, in the range of 3 to 3×10^2 units/l, were analysed in duplicate and in parallel with the tissue samples. After background correction, ^{14}C-oleic acid liberation of total radioactivity, was plotted on the x axis and the *Naja naja* venom PLA_2 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 $p < 0 \cdot 05$ considered significant. In the test of correlation, Spearman's correlation coefficient was used and $p < 0 \cdot 05$ considered significant.

Results

GROUP II PLA_2 mRNA EXPRESSION IN THE RAT GASTROINTESTINAL TRACT

Group II PLA_2 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 PLA_2 mRNA EXPRESSION

In both the glandular stomach and the distal part of ileum, the type II PLA_2 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 PLA_2 type I mRNA expression was not increased after endotoxin treatment (Table). Type I PLA_2 mRNA was not detected in the ileum, either before or after endotoxin treatment (data not shown).

EFFECT OF DEXAMETHASONE ON THE PLA_2 mRNA EXPRESSION

Twelve hours after the intravenous injection of endotoxin, the expression of PLA_2 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 PLA_2 type II mRNA expression after 12 hours was reduced both in the glandular stomach (Fig 2) and in the ileum

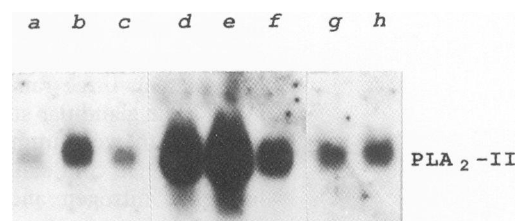

Figure 1: Northern blot analysis of group II PLA_2 mRNA (corresponding to 0·8 kb), in the rat gastrointestinal tract. Lane a, forestomach; b, glandular stomach; c, duodenum; d, ileum; e, distal ileum; f, caecum; g, proximal colon; h, distal colon. Each lane was loaded with 30 μg total RNA and the nylon filter hybridised with a rat group II PLA_2 oligonucleotide probe.

*PLA_2 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
PLA_2	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)
PLA_2	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).

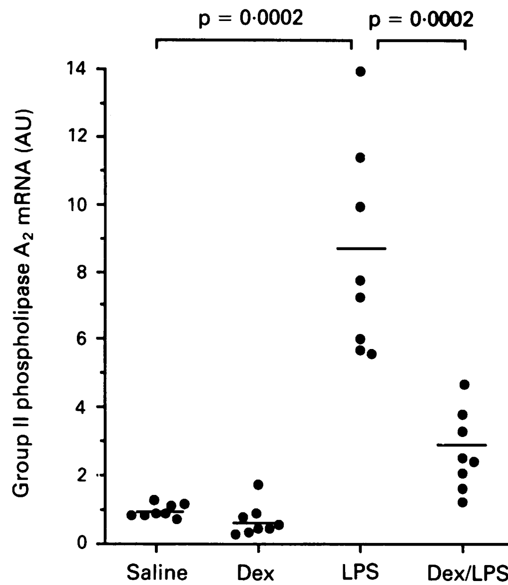

Figure 2: Group II PLA_2 mRNA expression in the rat glandular stomach 12 hours after challenge with lipopolysaccharide from E coli *(LPS) and in animals given dexamethasone 30 minutes before the endotoxin challenge (Dex/LPS). Saline alone (saline) and dexamethasone alone (Dex), respectively, was injected 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).*

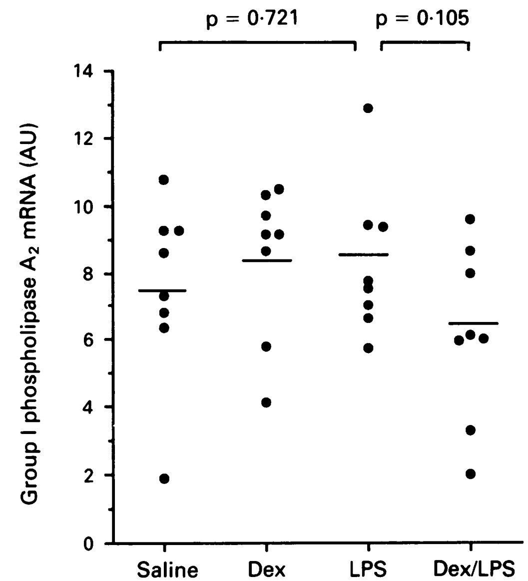

Figure 4: Group I PLA_2 mRNA expression in the rat glandular stomach 12 hours after challenge with lipopolysaccharide from E coli *(LPS) and in animals given dexamethasone 30 minutes before the endotoxin challenge (Dex/LPS). Saline alone (Saline) and dexamethasone alone (Dex), respectively, was injected 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 PLA_2 type II mRNA in the stomach (Fig 2) or ileum (Fig 3). Endotoxin did not affect the glandular stomach type I phospholipase A_2 mRNA expression, and dexamethasone was without effect (Fig 4).

EFFECT OF ENDOTOXIN AND DEXAMETHASONE ON THE PLA_2 ACTIVITY

The ileal PLA_2 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 PLA_2 activity, on the other hand, was not affected by endotoxin or dexamethasone (Fig 6).

CORRELATION ANALYSIS OF PLA_2 ENZYME ACTIVITY AND GROUP II PLA_2 mRNA EXPRESSION IN ILEUM

Spearman correlation analyses based on all groups – that is, including 32 animals, showed good correlation in ileal PLA_2 enzyme activity *v* group II PLA_2 mRNA expression (coefficient, $r=0{\cdot}73$; $p<0{\cdot}0001$) (Fig 7).

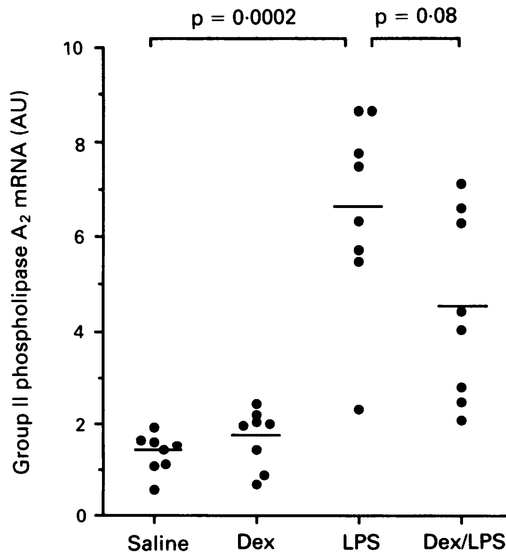

Figure 3: Group II PLA_2 mRNA expression in the rat distal ileum 12 hours after challenge with lipopolysaccharide from E coli *(LPS) and in animals given dexamethasone 30 minutes before the endotoxin challenge (Dex/LPS). Saline alone (Saline) and dexamethasone alone (Dex), respectively, was injected 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).*

Discussion

We have previously found that an increase in mucosal PLA_2 activity precedes the recurrence of intestinal inflammation in Crohn's disease.[5 6] This finding suggests the possibility that a group II PLA_2 might play a part in Crohn's disease, because group II phospholipases A_2 seem to participate in other inflammatory conditions[23 24]

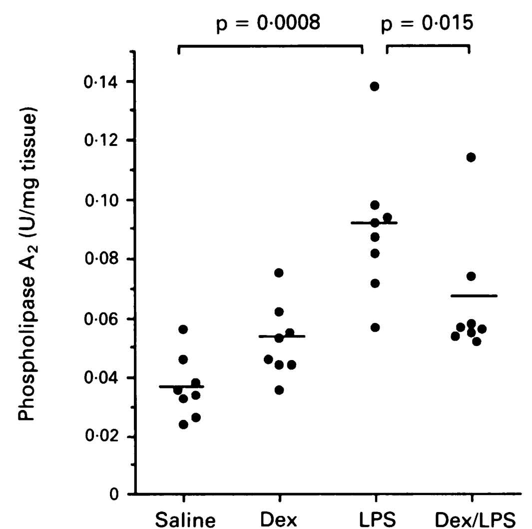

Figure 5: PLA_2 enzyme activity in the rat distal ileum 12 hours after challenge with lipopolysaccharide from E coli *(LPS) and in animals given dexamethasone 30 minutes before the lipopolysaccharide challenge (Dex/LPS). Controls were given saline alone (Saline) and dexamethasone alone (Dex), respectively. Each point represents the value from one animal (eight in each group).*

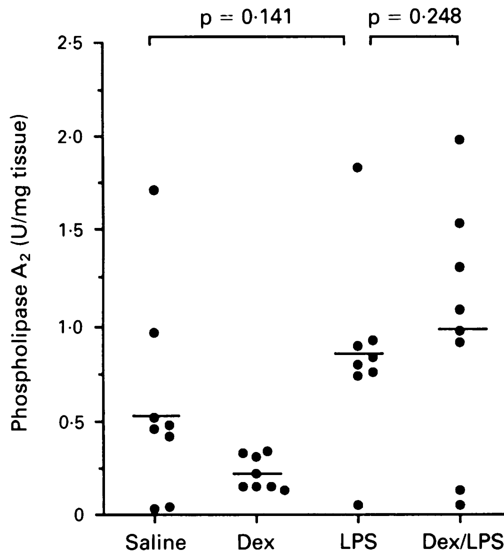

Figure 6: PLA_2 enzyme activity in the rat glandular stomach 12 hours after challenge with lipopolysaccharide from E coli (LPS) and in animals given dexamethasone 30 minutes before the lipopolysaccharide challenge (Dex/LPS). Controls were given saline alone (Saline) and dexamethasone alone (Dex), respectively. 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 PLA_2 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 PLA_2 mRNA in rats, and that the ileal expression of group II PLA_2 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 PLA_2 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 PLA_2 mRNA expression, but not the group I PLA_2 mRNA expression, was increased in the glandular stomach after endotoxin, suggesting that the expression of group II and group I PLA_2 mRNA is regulated differently in this tissue.

It has previously been shown that endotoxin stimulates intestinal mucosal PLA_2 activity in rats as soon as three minutes after an intravenous injection,[30] suggesting that endotoxin may stimulate intestinal PLA_2 activity also without previous induction of mRNA synthesis. It seems probable, therefore, that endotoxin induces intestinal PLA_2 activity by stimulating both post-transcriptional and pre-transcriptional events. Although the precise mechanisms, however, participating in the endotoxin stimulated PLA_2-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 PLA_2 activity[15 25 32-36] and PLA_2-II mRNA expression[15 25 37] in different cell types.

This study also showed that endotoxin increases the PLA_2 activity of the distal ileum. This is in agreement with previous investigations, showing that endotoxin may increase not only the PLA_2-II mRNA expression, but also the PLA_2-II activity.[15 25] Our results thus suggest that endotoxin may stimulate ileal group II PLA_2 activity by increasing the gene expression for this enzyme. Although the distal ileum is known to contain more than one type of PLA_2,[10 38] a correlation analysis based on all groups – that is, including 32 animals, shows a very strong correlation between the ileal group II PLA_2 mRNA activity and the PLA_2 activity (Fig 7). Thus, animals with a high ileal group II PLA_2 mRNA activity also had a high ileal PLA_2 activity. One has to assume, therefore, that the measured ileal PLA_2 activity mainly originated from group II PLA_2. The PLA_2 activity of the glandular mucosa, on the other hand, was not affected by endotoxin despite the increased PLA_2-II mRNA activity. One explanation might be a high level of group I PLA_2 activity in the glandular stomach,[8 14] as the group I PLA_2 mRNA expression was not affected by endotoxin. Moreover, the basal PLA_2 activity was about 10 times higher in the stomach than in the ileum, suggesting that minor changes of the PLA_2-II activity in the stomach were masked by the high PLA_2-I activity.

Pretreatment with the anti-inflammatory glucocorticoid, dexamethasone, reduced the endotoxin stimulated increase in group II PLA_2 mRNA, both in the ileum and the stomach, whereas the basal activity of group II PLA_2 mRNA, and the group I PLA_2 mRNA of the stomach, were unaffected. Moreover, dexamethasone reduced the PLA_2 activity of the ileum. These findings suggest that dexamethasone specifically inhibited the endotoxin induced increases in group II PLA_2 mRNA and activity. This is in agreement with previous investigations, showing that dexamethasone inhibits group II PLA_2 expression and activity in the aorta, spleen, lung, and thymus of endotoxin shock rats[19] 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 PLA_2 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 PLA_2-II mRNA by reducing the level

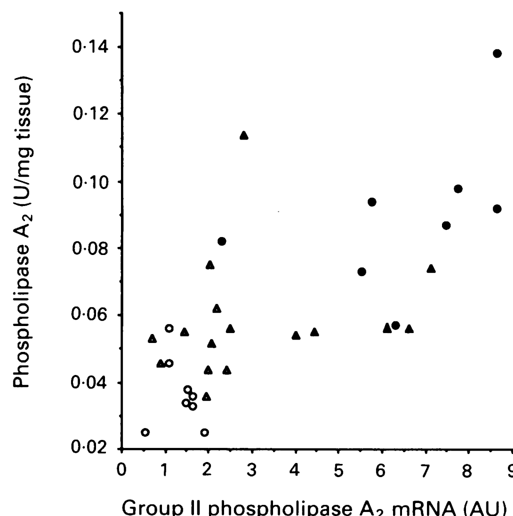

Figure 7: Scatter diagram illustrating the correlation between group II PLA_2 mRNA expression (x axis) and PLA_2 enzyme activity (y axis) in rat distal ileum. Correlation coefficient (including 32 animals), $r=0.73$; $p<0.0001$. Data from Figures 3 and 5. Challenge with lipopolysaccharide from E coli (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 PLA_2-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 PLA_2 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 PLA_2 activity and the group I PLA_2 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 PLA_2 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 PLA_2 activity and PLA_2 mRNA expression. This problem could be considered by studying the gastrointestinal PLA_2 mRNA expression after cell fractionation to yield homogenous cell populations or by in situ hybridisation. Moreover, to clarify the role of phospholipase A_2 in Crohn's disease, further studies are needed to elucidate the distribution and role of group I and II PLA_2 in the normal and diseased human gastrointestinal tract.

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