

Pathophysiological role of secretory type I and II phospholipase A₂ in acute pancreatitis: an experimental study in rats

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Abstract

Background—In human acute pancreatitis two different types of secretory phospholipase A₂ (PLA₂) have been found.

Aim—To analyse the specific pattern of distribution of these PLA₂ activities and their pathophysiological role in experimental acute pancreatitis.

Subjects and Methods—Catalytic activities of secretory type I (pancreatic) and type II (non-pancreatic) PLA₂ and the protein concentration of immunoreactive pancreatic PLA₂ (IR-PLA₂) in serum and pancreatic tissue of rats with cerulein (mild form) and sodium taurocholate (severe form) induced acute pancreatitis were determined.

Results—Cerulein infusion caused a significant increase in type I PLA₂ activity ($p < 0.01$) and IR-PLA₂ protein concentration ($p < 0.01$) in serum and pancreas, whereas type II PLA₂ activity remained unchanged during the 12 hour observation period. Histology showed no significant tissue destruction. In sodium taurocholate induced acute pancreatitis type II PLA₂ activity significantly increased, reaching values over 10-fold higher than controls ($p < 0.01$), whereas IR-PLA₂ protein concentration and type I PLA₂ activity were only marginally increased. In this severe model of acute pancreatitis significantly lower values were detected than in the control pancreas ($p < 0.002$) for PLA₂ activity and IR-PLA₂ protein concentration. Histology showed parenchymal and fat necroses with haemorrhage, oedema, and inflammatory cell infiltration.

Conclusions—Type I PLA₂ activity is dependent on the IR-PLA₂ protein concentration in serum and pancreatic tissue. The type II PLA₂ activity is not stimulated by cerulein, which indicates an extra-acinar origin of this enzyme. Type II PLA₂ activity is significantly increased in sodium taurocholate induced acute pancreatitis indicating its role in the local necrotising process and involvement in the systemic effects in severe acute pancreatitis.

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Keywords: acute pancreatitis, phospholipase A₂, prognosis, cerulein pancreatitis, sodium taurocholate pancreatitis.

The hypothesis that the lipolytic enzyme phospholipase A₂ (PLA₂) which catalyses the hydrolysis of phosphoglycerides at the sn-2-position¹ plays an important part in inflammatory diseases, has been postulated by many authors. Increased catalytic PLA₂ activities have been detected in septic shock,²⁻⁴ rheumatoid arthritis,⁵ acute pancreatitis,⁶⁻⁷ peritonitis,^{3, 8} multiple injuries,^{8, 9} pulmonary diseases such as acute respiratory distress syndrome,^{3, 4, 6, 14} and recently in patients with cancer.⁷

Two types of human secretory PLA₂ have been described: (a) the pancreatic PLA₂ (type I PLA₂), with a disulphide bridge between cysteine at position 11 and cysteine at position 77, secreted by acinar cells, and (b) the non-pancreatic PLA₂ (type II PLA₂) of unknown source which lacks these cysteine residues and the corresponding disulphide bridge.¹¹⁻¹⁴ The question arises as to which type of PLA₂ plays the crucial part in acute pancreatitis. Different methods such as time resolved fluorimmunoassays and radioimmunoassays have been developed for the determination of the protein concentrations of type I and type II PLA₂.¹⁵⁻¹⁷ In addition, photometric and radioenzymatic assays with different substrates (¹⁴C labelled synthetic phospholipids or *Escherichia coli* membranes) are sufficiently sensitive for the measurement of the catalytic activity of PLA₂.^{18, 19} Immunoabsorption studies and clinical studies which compared immunoreactive pancreatic PLA₂ protein concentration and total catalytic PLA₂ activity in serum from patients with inflammatory diseases indicated an extrapancreatic source of PLA₂.^{8, 9, 20, 21} In the past, it was not possible to measure the pancreatic type I and extrapancreatic type II PLA₂ activities separately. For this reason, the diagnostic and prognostic implications of PLA₂ in acute pancreatitis have remained speculative. The aim of the present study was to characterise the pathophysiological role of the two secretory types of PLA₂ in both mild and severe forms of experimental acute pancreatitis.

Methods

Study design

Approval for the experiments was obtained from the animal ethics committee of the University of Bern. Thirty female Wistar rats

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weighing 260–320 g were subdivided into three experimental groups with 10 animals each.

Model of oedematous acute pancreatitis: cerulein pancreatitis – The model of hyperstimulation of pancreatic secretion by the pancreatic secretagogue cerulein²² was used to simulate oedematous or mild acute pancreatitis.

All animals had free access to food and water before and during the experiments. All operations were performed under halothane anaesthesia (Fluothane, ICI GmbH Germany, concentration: 0.5–3.5%, delivery rate: 1.8 lO₂/min) using a sterile technique.²³ To manage postoperative pain buprenorphine (Temgesic, Boehringer Mannheim GmbH, Germany) was applied subcutaneously at a dosage of 0.01 mg/100 g body weight every six hours.

After preparation and cranial ligation of the right external jugular vein, a silicon catheter (Down Corning GmbH, Meerbusch, Germany; outside diameter 1.65 mm) was implanted in the vessel and the free end of the catheter was brought through the skin at the back of the neck. This venous catheter was used for cerulein infusion (Takus, Farmitalia Carlo Erba GmbH, Bremen, Germany) which was performed at a rate of 0.6 ml/h for two hours and at a dosage of 5 µg/kg body weight/hr and for blood sampling immediately before cerulein administration and at 3, 6, and 12 hours after the beginning of infusion. Each blood sample (0.7 ml) was substituted with a similar volume of saline (37°C). Between the surgical intervention and the induction of acute pancreatitis the animals were allowed to recover for 12 hours.

Model of necrotising acute pancreatitis: sodium taurocholate pancreatitis – In this group acinar cell damage (necrotising acute pancreatitis) was induced by pressure controlled intraductal injection of 3% sodium taurocholate solution.²⁴

Catheterisation of the jugular vein was performed in 10 animals as described above. Laparotomy was performed by a midline incision and the duodenal part of the pancreatic gland was exposed. The biliopancreatic duct was cannulated transmurally with a 27 G needle. The biliary duct was closed with a bulldog clamp placed distally to the pancreas at the hilum of the liver. Thereafter 3% sodium taurocholate solution (Na taurocholate, Sigma Chemical Co, St Louis, USA) with a dosage of 0.1 ml/100 g body weight was infused under constant pressure (30 cm H₂O). After the administration of taurocholate, the clamp and needle were removed and the abdominal wall was closed. Blood samples were taken before the induction of acute pancreatitis and at 3, 6, 12, 18, and 24 hours afterwards via the implanted venous catheter.

Controls – This third group consisted of 10 untreated rats which served as controls for serum and tissue analyses.

At the end of the observation period (cerulein: 12 hours, taurocholate: 24 hours) the animals were again anaesthetised and the whole pancreatic gland was removed. Parts of the biliary segments were taken for histological

examination. These were fixed in Bouin's solution (picric acid, formalin 37%, 3:1), and dehydrated in ethanol (70%). The remainder of the tissue was frozen in dry ice and stored at –80°C for subsequent measurements of immunoreactive pancreatic PLA₂ protein concentration (IR-PLA₂), total PLA₂ activity, and type I and type II PLA₂ activity, at which time it was lyophilised, homogenised in Tris buffer and centrifuged at 20000 rpm for 45 minutes.²⁵ The supernatant was then taken for biochemical analysis.

Biochemical analysis

Serum and tissue immunoreactive pancreatic PLA₂ protein concentrations (IR-PLA₂) were measured with a time resolved fluoroimmunoassay. The lowest concentration measurable by the assay is 0.25 ng/ml. After addition of the samples (25 µl) and assay buffer (175 µl; Wallac, Turku, Finland), the microtitre plate coated with monoclonal antipancreatic PLA₂ antibodies was incubated for 60 minutes and then washed with a solution of NaN₃ (0.5 g/l in saline). A further incubation was performed at room temperature after the addition of 200 µl europium labelled antirat pancreatic PLA₂ antibodies. Fluorescence was measured with an Arcus fluorometer (Wallac, Turku, Finland) at 613 nm, 10 minutes after addition of enhancement solution (200 µl per tube). The production of antirat pancreatic PLA₂ antibody, PLA₂ standards, microtitration plates coated with antirat pancreatic PLA₂ antibody, and europium labelled antirat pancreatic PLA₂ antibody are described elsewhere.²⁶

Total catalytic PLA₂ activity and type I and type II PLA₂ activities were measured with an *E coli* assay, based on the hydrolysis of phospholipids from [¹⁴C] oleic acid labelled *E coli* biomembranes in the sn-2 position.¹⁸ Briefly, the reaction mixtures of lyophilised labelled *E coli* biomembranes, Tris HCl, and fatty acid free bovine serum albumin (RPRA HCl: pH 8.0, bovine albumin 5 g, Tris 3.03 g, CaCl 0.18 g, NaCl 0.11 g), and the serum or tissue samples were incubated (37°C, 15 minutes). The reaction was stopped with EDTA and the lipids were extracted with propan-2-ol/n-heptane/1 M H₂SO₄ (40:10:1). The organic phase of the solution was passed through an aminopropyl column. The unhydrolysed substrate remains on the column while the free radioactive oleates pass through. The eluant from the column was collected and its radioactivity was measured with a β-scintillation counter (Betamatic I, Kontron Instruments Zürich, Switzerland). The total catalytic activity of PLA₂ was determined in untreated serum samples. The differentiation of the total catalytic PLA₂ activity into type I PLA₂ and type II PLA₂ activity was done using the different physiological properties of these enzymes. In contrast with extrapancreatic PLA₂, pancreatic PLA₂ is heat stable (60°C for 60 minutes).^{12, 13} Aufenanger *et al*²⁷ showed the efficacy and specificity of the *E coli* assay in human acute pancreatitis in differentiating type I and II PLA₂ activities: after heat inacti-

Protein concentration of immunoreactive pancreatic PLA₂ (IR-PLA₂) and lipase activity in cerulein and sodium taurocholate induced acute pancreatitis

	Time (hr)						p Values v basal levels (0 hr)
	0	3	6	12	18	24	
	<i>IR-PLA₂ (ng/ml)</i>						
Cerulein	20.4 (20.0; 22.6)	*3355 (2920.5; 3535.7)	*305.4 (180.0; 535.5)	*53.22 (42.2; 78.0)	—	—	*<0.01
Taurocholate	22.4 (19.2; 23.8)	*665.6 (568.0; 743.6)	*187.3 (181.0; 199.2)	*136.5 (89.4; 142.0)	*57.9 (35.3; 71.5)	35.1 (28.0; 37.4)	*<0.02
Significance	NS	p<0.002	NS	p<0.01	—	—	
	<i>Lipase activity (U/l)</i>						
Cerulein	80 (40; 100)	*4540 (4165; 5675)	*3280 (1975; 4235)	*1350 (710; 1520)	—	—	*<0.01
Taurocholate	50 (40; 65)	*3020 (2145; 4110)	*1210 (960; 1560)	*470 (385; 950)	*200 (170; 305)	120 (75; 150)	*<0.02
Significance	NS	NS	p<0.01	NS	—	—	

Values are medians (lower; upper quartiles).

vation of non-pancreatic PLA₂ and after subsequent absorption of pancreatic PLA₂ with a monoclonal antibody against human pancreatic PLA₂, no PLA₂ activity was detectable in serum samples. Therefore, the heat stable type I PLA₂ activity was measured in serum samples after heat treatment at 60°C for 60 minutes by the *E coli* method, and type II PLA₂ activity was calculated by the subtraction of type I PLA₂ from total PLA₂ activity (type II PLA₂=total PLA₂-type I PLA₂).

Serum lipase activity was determined by an enzymatic assay which is commercially available from Boehringer Mannheim, Germany.

Histology

The formalin fixed tissue was embedded in paraffin, cut in 5 µm thick sections, and stained with haematoxylin and eosin. The histological findings were analysed using the method developed by Spormann *et al.*²⁸ Briefly, oedema, parenchymal necrosis, fatty tissue necrosis, inflammatory infiltration, and haemorrhage were graded (scale: 1 to 3) in a blinded fashion. At the end, the mean total score of each histopathological variable was calculated.

Statistics

As our data were not normally distributed, all results are given as medians and upper and lower quartiles. The differences between the groups were tested with the Wilcoxon test for

paired and unpaired samples. The correlation between two variables was tested by linear regression analysis, and p<0.05 was taken as significant.

Results

Serum protein concentration of immunoreactive pancreatic PLA₂

In cerulein infused rats, the rise in concentration of type I PLA₂ from the basal median 20.4 ng/ml to 3355 ng/ml at three hours was highly significant (p<0.01). The three hour peak decreased rapidly thereafter (Table). A positive correlation (r=0.78; p<0.0001) between pancreatic type I PLA₂ activity and IR-PLA₂ protein concentration was found (Fig 1).

In sodium taurocholate induced acute pancreatitis, the three hour concentration of IR-PLA₂ was significantly lower than in cerulein induced acute pancreatitis (665.6 v 3355 ng/ml; p<0.002) and thereafter the concentration of IR-PLA₂ fell slowly to normal. In this necrotising model of acute pancreatitis, similar results were found for type I PLA₂ activity and IR-PLA₂ protein concentration, although the correlation was less strong (r=0.6; p<0.001, Fig 1). The IR-PLA₂ concentration and pancreatic type I PLA₂ activity also correlated well with lipase activity in both models (IR-PLA₂ v lipase, cerulein model: r=0.68, p<0.0004; taurocholate model: r=0.67, p<0.0004; type I PLA₂ activity v lipase cerulein model: r=0.65,

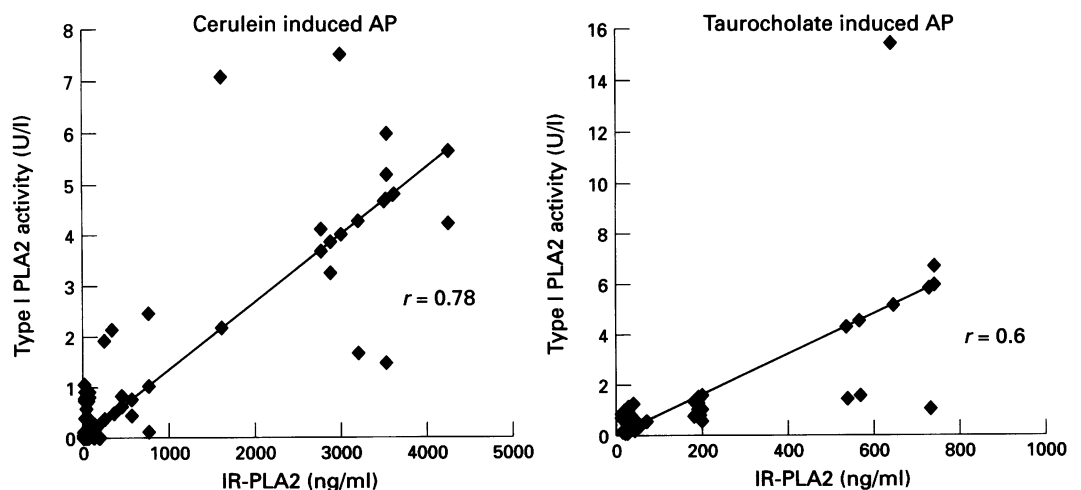


Figure 1: Linear regression analysis between the immunoreactive pancreatic IR-PLA₂ protein concentration and the activity of type I PLA₂ in serum samples from rats with cerulein induced acute pancreatitis (r=0.78, p<0.0001) and sodium taurocholate induced acute pancreatitis (r=0.6, P<0.001). AP=acute pancreatitis.

$p < 0.0001$; taurocholate model: $r = 0.76$, $p < 0.0001$) (Table).

Serum catalytic PLA₂ activity

Figs 2 and 3 show the time courses for serum catalytic activities of type I and type II PLA₂ and total catalytic PLA₂ activity, after onset of cerulein and taurocholate induced acute pancreatitis. No type I PLA₂ activity was detected in either group before the induction of acute pancreatitis. Peak total catalytic PLA₂ activity occurred at three hours after cerulein infusion ($p < 0.01$ v basal median, Fig 2) and thereafter fell towards normal concentrations. The rise in PLA₂ activity was mainly due to the increase in pancreatic type I PLA₂ activity in the cerulein group. In taurocholate induced acute pancreatitis (Fig 3) the three hour peak was caused by the increase in type II PLA₂ activity, which reached values over 10-fold above normal ($p < 0.01$) and significantly higher ($p < 0.002$) than after cerulein infusion.

In contrast, in cerulein infused rats the non-pancreatic type II PLA₂ was not significantly raised during the 12 hour observation period (Fig 2) and remained constant during the experiment. The three hour peak for pancreatic type I PLA₂ activity in the cerulein model was fivefold higher than the rise in type I PLA₂ activity in taurocholate induced acute pancreatitis at this time ($p < 0.002$).

Tissue analysis and histology

By comparison with control animals type I PLA₂ activity and IR-PLA₂ protein concentration in pancreatic tissue of cerulein induced acute pancreatitis were significantly increased

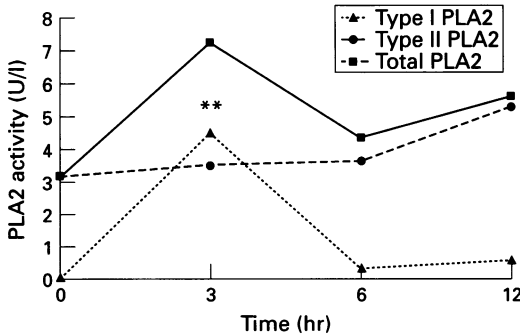


Figure 2: Cerulein induced acute pancreatitis. Activities of type I, type II, and total PLA₂; medians of serum sample values, ** $p < 0.01$; v basal values.

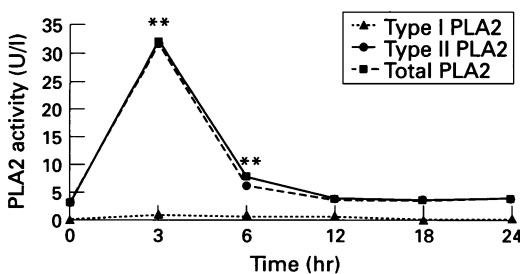


Figure 3: Sodium taurocholate induced acute pancreatitis. Activities of type I, type II, and total PLA₂; medians of serum sample values; ** $p < 0.01$ v basal values.

12 hours after induction of acute pancreatitis (controls v cerulein: type I activity: 0.8 v 23.4 U/g, $p < 0.002$; IR-PLA₂: 500 v 1120 ng/mg, $p < 0.05$; Figs 4, 5). Similar activities for type II PLA₂ in pancreatic tissue were found in both the controls and cerulein induced acute pancreatitis (type II PLA₂: 36.1 v 40.1 U/g). However, in sodium taurocholate acute pancreatitis, type I and type II PLA₂ activities and IR-PLA₂ protein concentrations were significantly reduced compared with controls (controls v taurocholate: type I PLA₂: 0.8 v 0.15 U/g; type II PLA₂: 36.1 v 14.8 U/g, $p < 0.002$; IR-PLA₂: 500 v 308.4 ng/mg, $p < 0.002$; Figs 4, 5).

The histological analysis of pancreatic gland destruction showed significantly higher scores ($p < 0.002$) of all examined variables in taurocholate induced acute pancreatitis by comparison with cerulein induced acute pancreatitis (Fig 6).

Discussion

Cerulein infusion in rats was characterised by a pronounced increase in total serum catalytic PLA₂ activity but with a largely undamaged pancreatic gland which showed only mild, oedematous histological alterations. Sodium taurocholate induced acute pancreatitis proved to be more severe, with extensive acinar cell and fat necroses, inflammatory infiltration, and haemorrhage, as has been described by other authors.^{22 24}

In the cerulein model, serum secretory type II (non-pancreatic) PLA₂ showed no change in activity within the 12 hour observation period. By contrast, the immunoreactive pancreatic protein concentration (IR-PLA₂) and the type I (pancreatic) PLA₂ activity in serum before cerulein infusion, increased rapidly on its administration with peak activity occurring at three hours. The resultant fall in activity was probably due to the elimination of PLA₂ from circulation by the kidneys²⁹ as well as the short duration (two hours) of hyperstimulation.

Several reports have indicated that a low infusion dose of cerulein can stimulate the exocrine cells of the pancreas to secrete proteins and to activate secretory steps such as protein synthesis and granule luminal discharge; whereas it would seem that high doses alter the luminal to lateral exocytosis with

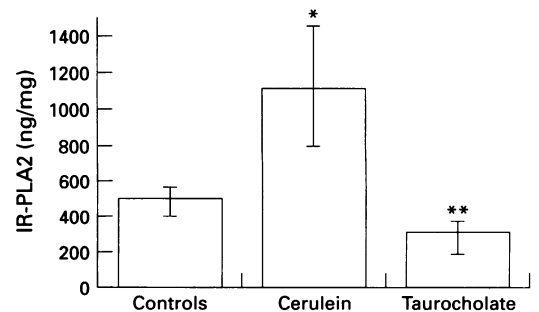


Figure 4: Protein concentration of pancreatic IR-PLA₂ in controls and cerulein and sodium taurocholate induced acute pancreatitis; medians and quartiles in pancreatic tissue samples after the observation periods (cerulein: 12 hr, sodium taurocholate: 24 hr); * $p < 0.05$; ** $p < 0.002$.

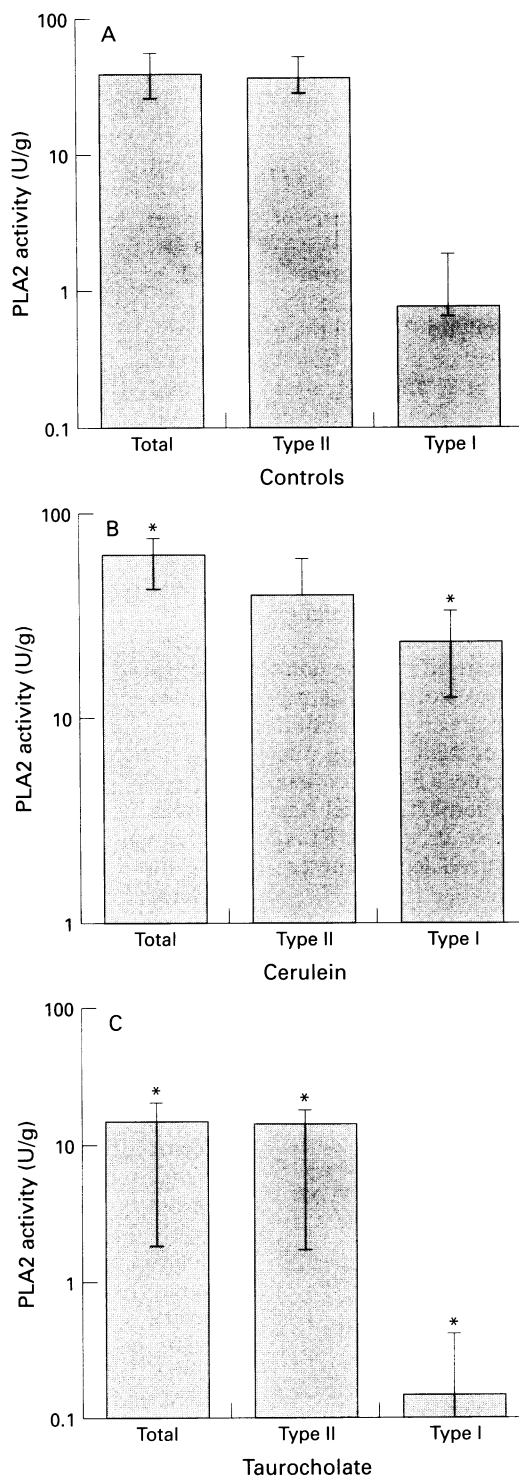


Figure 5: Comparison of changes in total PLA₂ activity and the activities of type I and type II PLA₂ between controls, cerulein, and sodium taurocholate models: medians in pancreatic tissue samples after the observation periods (cerulein: 12 hr, taurocholate: 24 hr); * $p < 0.002$ v controls.

discharge of secretory enzymes like PLA₂ into the interstitial space, resulting in inflammation.^{22 30 31} Lymph drainage and venous and peritoneal resorption^{32 33} of the released enzymes or proenzymes are also thought to have roles in the rise in serum pancreatic IR-PLA₂ protein concentration, type I PLA₂ activity, and the lipase activity in cerulein infused rats. The same mechanisms are responsible for resorption of the enzymes released through

acinar cell destruction during sodium taurocholate induced acute pancreatitis.

It has been reported that there is no correlation between the total PLA₂ activity and pancreatic IR-PLA₂ protein concentration in human and experimental acute pancreatitis.^{25 34} The results of this investigation supported these findings and furthermore showed similar courses for pancreatic IR-PLA₂ protein concentration and type I PLA₂ activity in blood with a positive correlation between them in both the cerulein and sodium taurocholate model, once total catalytic PLA₂ activity was differentiated into type I and type II PLA₂ activities. In both models of acute pancreatitis, type I PLA₂ activity, pancreatic IR-PLA₂ protein concentration, and lipase activity in serum correlated significantly with each other, but not with non-pancreatic type II PLA₂ activity. These results indicate that the measured activity of type I PLA₂ is due to the presence of pancreatic PLA₂.

We found large changes in the activity of the type II PLA₂, three hours after intraductal sodium taurocholate administration. In serum, the activity of this enzyme rose 10-fold above normal, which is comparable with the increase in total catalytic PLA₂ activity in severe acute pancreatitis in humans.^{6 34} Our results indicate that the serum course for total PLA₂ activity was caused by an increase in non-pancreatic type II PLA₂ in this severe form of experimental acute pancreatitis. Type I PLA₂ activity did not change significantly with taurocholate, an obvious indication of its "non-inflammatory" role in this disease. Kawai *et al*³⁵ reported similar findings for 5% sodium taurocholate solution and a similar method of PLA₂ activity measurement. Furthermore, it has been reported that in patients with necrotising acute pancreatitis, PLA₂ activity seems to correlate significantly with the concentration of extra-pancreatic type II PLA₂ and with the severity of the disease but not with the protein concentration of pancreatic IR-PLA₂.^{6 34} These findings and our own results support the hypothesis that raised PLA₂ activity in human and experimental necrotising acute pancreatitis is caused by an increase in non-pancreatic type II PLA₂ activity. Moreover, the "non-stimulation" of type II PLA₂ in cerulein infused rats is indicative of its extra-acinar origin.

Protein synthesis, inhibition of secretion, and storing of pancreatic IR-PLA₂ proenzyme are probably the mechanisms responsible for the increased tissue protein concentrations of pancreatic IR-PLA₂ in cerulein-infused rats.^{22 30} Accordingly, the raised tissue values of type I PLA₂ activity are due to the presence of pancreatic IR-PLA₂. The decreased tissue pancreatic IR-PLA₂ protein concentration and type I and II PLA₂ activities, 24 hours after the induction of taurocholate induced acute pancreatitis, are perhaps due to denaturation and inactivation of the enzyme in the necrotic and acid milieu as would seem to be the case in human acute pancreatitis.³⁶ Lysolecithin as a conversion product of the lecithin catalysing PLA₂ can indirectly reflect the presence of PLA₂ activity. Increased lysolecithin concen-

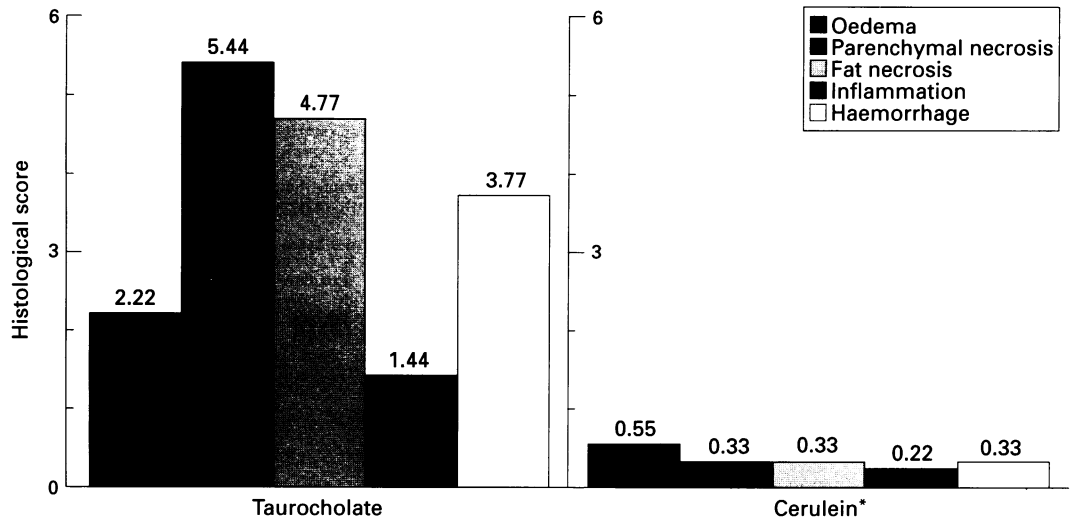


Figure 6: Quantitative histological analysis of the pancreas stained with haematoxylin and eosin. Comparison between cerulein and taurocholate induced acute pancreatitis; * $p < 0.002$ for all examined variables.

trations in pancreatic tissue samples from rats have been reported three to 12 hours after taurocholate induced acute pancreatitis, and return to the control value 24 hours after induction of acute pancreatitis.³⁷ These results also indicate the inactivation of the enzymatic activity of PLA₂ in rat tissue at a later stage of the inflammatory process. It should be noted that the measured PLA₂ activities in pancreatic tissue samples may be a reflection of the activation of inactive PLA₂ proenzymes by trypsin during the process of tissue homogenisation and the enzymatic assay.³⁸

Our findings lead to the conclusions that: (a) pancreatic type I PLA₂ activity is dependent on the protein concentration of pancreatic IR-PLA₂; (b) type I PLA₂ is non-toxic to acinar cells in both models of experimental acute pancreatitis (mild and severe forms) in the rat; (c) non-pancreatic type II PLA₂ is stimulated by an as yet undetermined process in sodium taurocholate induced acute pancreatitis of extra-acinar origin; and (d) the involvement of non-pancreatic PLA₂ in both human and experimental necrotising acute pancreatitis plays a dominant pathophysiological part in this disease. Therefore, the specific inhibition of type II PLA₂ to prevent local and systemic complications of severe acute pancreatitis may well warrant further investigations.

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