Inhibition of Porcine Pancreas Phospholipase A₂ Activation by Gabexate Mesilate

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Summary. We investigated the effect of gabexate mesilate on the catalytic activity of phospholipase A₂ in homogenized porcine pancreatic tissue. Gabexate mesilate is a potent inhibitor of serine proteases. There is no direct inhibition of phospholipase A₂ catalytic activity in concentrations up to 6 mmol/l. Preincubation of homogenized pancreatic tissue with gabexate mesilate leads to a reduction of phospholipase A₂ activity even in concentrations as low as 6 μmol/l. The activation of purified porcine prophospholipase A₂ added to pancreatic tissue can be completely inhibited. Thus, gabexate mesilate might influence the activation of phospholipase A₂ administered in therapeutic concentrations in inflamed pancreatic tissue.

Key words: Phospholipase A₂ – Gabexate mesilate

Introduction
Gabexate mesilate (ethyl-p-(6-guanidinohecanoyloxy)-benzoate methanesulfonate) is a potent protease inhibitor. Possibly it reaches high concentrations in tissue due to its low molecular weight. In clinical studies continuous intravenous infusion of gabexate mesilate is applied to patients with acute pancreatitis to minimize the damage induced by trypsin, elastase, and other extrapancreatic proteases [4]. Prophospholipase A₂ (ProPLA₂) is stored in the zymogen granula of pancreas acinar cells and converted to the active enzyme by the tryptic cleavage of the N-terminal heptapeptide at the arginine-alanine linkage [5]. Active PLA₂ is thought to be a main agent of tissue damage resulting from the liberation of membrane toxic lysophospholipids [9]. It has been proposed that PLA₂ is also inhibited by gabexate mesilate [2]. However, PLA₂ catalytic activity is competitively inhibited by gabexate mesilate in high concentrations only (Kᵢ 500 μmol/l [2] and Kᵢ 130 μmol/l [6], depending on different substrates and substrate concentrations). But a complete inhibition of the trypsin activation of ProPLA₂ should be achievable already with lower concentrations of gabexate mesilate (Kᵢ 9.4 μmol/l for trypsin inhibition [7]). We investigated the effect of gabexate mesilate on homogenized porcine pancreatic tissue.

Materials and Method
Gabexate mesilate was a gift from Sanol (Schwarz, Monheim, FRG). Porcine phospholipase A₂ was purchased from Boehringer (Mannheim, FRG) and porcine prophospholipase A₂ from Calbiochem (La Jolla, USA). For the homogenization of pancreas tissue we used a Dismembrator (Braun, Melsungen, FRG).

Preparation of the Tissue Homogenate
Porcine pancreas was deep-frozen in liquid nitrogen immediately after slaughter. The deep-frozen tissue was homogenized in a Dismembrator for 30 s. Aliquots of the homogenate were stored at −70°C. The tissue homogenate was resuspended in isotonic saline solution. 1 g tissue per 10 ml solution. It was then ready for use.

Aliquots of the tissue preparation were preincubated with gabexate mesilate in concentrations varying from 6 μmol/l to 60 mmol/l. A 1:100 (vol/vol) dilution of ProPLA₂ was prepared and preincubated with the tissue preparation alone and with tissue preparation in the presence of gabexate mesilate. As a control experiment purified proenzyme...
and purified PLA₂ (1:50,000 vol/vol) were preincubated with gabexate mesilate. The determination of PLA₂ catalytic activity has been described elsewhere [8]. The assay was performed with 10 µl aliquots in duplicates.

Results

Figure 1 shows the liberation of palmitic acid over an incubation period of 35 or 40 min. The activity of the purified porcine pancreas PLA₂ is shown in curve a and the ProPLA₂ activity in curve b. Each symbol represents the mean of duplicates. The experiment was done in the absence (open symbols) and presence (filled symbols) of gabexate mesilate (60 µmol/l). In the purified enzyme and proenzyme preparations even this high inhibitor concentration revealed no diminished fatty acid liberation. The slopes of the curves were identical whether or not gabexate mesilate was present.

Gabexate mesilate at concentrations from 6 µmol/l to 6 mmol/l did not diminish PLA₂ activity. At a concentration of 60 mmol/l the reaction mixture was destabilized and no activity could be detected (not shown).

Pancreas tissue was highly active with regard to the liberation of palmitic acid; after 25 min the tissue activity decreased (Fig. 2a). After preincubation with gabexate mesilate in the rather low concentration of 60 µmol/l, the tissue activity of PLA₂ was markedly diminished to 1/3 (Fig. 2b). The reaction progress was linear throughout 45 min. A further reduction of PLA₂ activity by higher concentrations of gabexate mesilate was not achievable (not shown). The activity of the porcine proenzyme added to pancreas tissue was highly increased within the tissue, as shown in Fig. 2c. Its activity was markedly higher than the same ProPLA₂ preparation dissolved in isotonic NaCl solution (Fig. 1b). Preincubation with gabexate mesilate led to a six-fold reduction of activity (Fig. 2d).

Discussion

A common technique to detect PLA₂ activity in pancreas tissue preparations is the extraction and quantitative measurement of lysophospholipids.