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Contraction of guinea pig lung parenchyma by pancreatic type phospholipase A_2 via its specific binding site

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Porcine pancreatic group I phospholipase A_2 (PLA_2-I) induced contraction of guinea pig lung parenchyma in a concentration-dependent manner. Its EC_{50} value was similar to the K_d value calculated from the specific binding of ^{125}I-labeled porcine PLA_2-I in the membrane fraction of guinea pig lung. Type-specific action of PLA_2's and homologous desensitization strongly implicated the involvement of PLA_2-I-specific sites in the activation process. Thromboxane A_2 was found to be the main product from lung tissue by PLA_2-I action and the contractile response by PLA_2-I was specifically suppressed by thromboxane A_2 receptor antagonists and cyclooxygenase inhibitor, but not by leukotriene receptor antagonist and H1 blocker. These findings indicate that PLA_2-I-induced contractile response may depend on the secondarily produced thromboxane A_2, thus providing a new aspect of PLA_2-I from the pathophysiological standpoint.

Phospholipase A_2; Specific binding site; Thromboxane A_2; Guinea pig lung parenchyma; Contraction; (+)-S-145Na

1. INTRODUCTION

Mammalian extracellular phospholipase A_2 (PLA_2) can be classified into two types, pancreatic group I (PLA_2-I) and arthritic group II (PLA_2-II), based on their primary structures [1]. PLA_2-I is mainly secreted from the pancreas and has long been thought to be a digestive enzyme [2]. However, we recently found a protein specifically binding PLA_2-I (M_r ~ 190,000) in membranes of various cells and tissues including those of human origin [3,4]. We also reported that PLA_2-I directly stimulated DNA synthesis in Swiss 3T3 cells [4], rat synovial and vascular smooth muscle cells [3], and also induced chemokinetic migration in rat embryonic aortic smooth muscle cells (A7r5) [5]. These findings strongly implicate PLA_2-I in the modulation of cellular functions with respect to some pathophysiological states. Further evidence for the presence of PLA_2-I in several tissues, such as lung, kidney and small intestine [6–9], prompted us to examine PLA_2-I for other physiological functions in these tissues. In this study we have characterized the PLA_2-I-specific binding sites in guinea pig lung parenchyma and examined the effect of PLA_2-I from the pathological standpoint.

Abbreviations: PLA_2, phospholipase A_2; ACh, acetylcholine; TxB_2, thromboxane B_2; PGD_2, prostaglandin D_2; LT, leukotriene.

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2. MATERIALS AND METHODS

2.1. *Materials*

Preparation of various PLA_2's and iodination of porcine PLA_2-I were carried out as described previously [3,4]. The sodium salt of (+)-S-145 ((+)-S-145Na) [10], ONO3708 [11] and FPL55712 [12] were synthesized in our laboratories. Pyrilamine, indomethacin and acetylcholine chloride (ACh) were purchased from Sigma (St. Louis, MO).

2.2. *Crude membrane preparation from guinea pig lung tissue*

Male guinea pigs weighing 300–600 g (Charles River Japan Inc., Kanagawa, Japan) were sacrificed by stunning and exsanguination. The lungs were removed and homogenized in 4 vols. of buffer A (20 mM Tris/HCl, pH 7.4, 2 mM EDTA, 0.4 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin and 0.25 M sucrose). The homogenate was centrifuged at 3,000 × *g* for 20 min. The supernatant was then centrifuged at 105,000 × *g* for 60 min. The pellets were resuspended in buffer A, dispersed by gentle homogenization, and stored at −80°C until assay.

2.3. *Binding experiments*

The binding experiments were performed by incubating lung membranes with various concentrations of porcine [^{125}I]PLA_2-I in a total volume of 0.2 ml of buffer B (20 mM Tris/HCl, pH 7.4, 2 mM EDTA and 0.1% BSA) at 4°C for 2 h. Specific binding was defined as the difference between binding in the presence and absence of the unlabeled porcine PLA_2-I (500 nM). After the incubation the radioactivity in the membrane was analyzed as reported previously [4]. The specific binding was 85% of the total binding. Competition experiments were performed by incubating the membranes with 3 nM porcine [^{125}I]PLA_2-I in the presence of 100 nM of PLA_2's under similar binding conditions.

2.4. *Contractile activity on guinea pig lung parenchymal strips*

Lungs from guinea pigs were removed and perfused with Kreb's solution (composition, mM: NaCl 118.0; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $NaHCO_3$ 25.0; $MgSO_4 \cdot 7H_2O$ 1.2; and glucose 10.0) until free of blood. Lung parenchymal strips were carefully cut from the distal edges of the lower lung lobes (approximately 3 × 1 × 20 mm) and

suspended in an organ bath containing 10 ml of Kreb's solution maintained at 37°C and gassed continuously with 95% O_2 and 5% CO_2. Tissue contractions were recorded isometrically using force transducers (Nihon Kohden TB611T, Japan). The tissues were allowed to equilibrate for at least 60 min under a resting tension of 0.5 g, and then constant maximal contractions to ACh (1 mM) were obtained before the additions of porcine PLA_2-I at varying concentrations. For pharmacological studies on porcine PLA_2-I-induced contractions, the strips were pre-incubated with several drugs before adding PLA_2-I. To minimize inter-tissue variability the contractile response was expressed relative to the contraction elicited by the 1 mM ACh.

2.5. *Porcine PLA_2-I-induced mediator synthesis and release from guinea pig lung tissue*

Lungs were removed and rinsed in Kreb's solution then minced into 1–2 mm^3 pieces with a razor. The minced lung (0.1 g/ml) was incubated with porcine PLA_2-I at 10 nM (unless otherwise stated) or vehicle in Kreb's solution at 37°C for 1–30 min. The concentrations of thromboxane B_2 (TxB_2), prostaglandin D_2 (PGD_2) and leukotriene (LT) $C_4/D_4/E_4$ in the supernatant were measured by radioimmunoassay, and that of histamine was determined by HPLC [13].

2.6. *Statistics*

Statistical analysis was performed using Student's *t*-test.

3. RESULTS

At various concentrations porcine PLA_2-I specifically bound to crude guinea pig lung membranes, of which the level of specific binding was about 85% of total binding. The Scatchard plot of the specific binding (Fig. 1) is linear, indicating the presence of a single class of binding site with a K_d value of 3.3 nM and a maximal binding capacity (B_{max}) of 46.0 fmol/mg protein. This K_d value is similar to those reported for other cells and tissues that possess specific PLA_2-I binding sites [3]. The specific binding of [^{125}I]PLA_2-I to the lung membranes

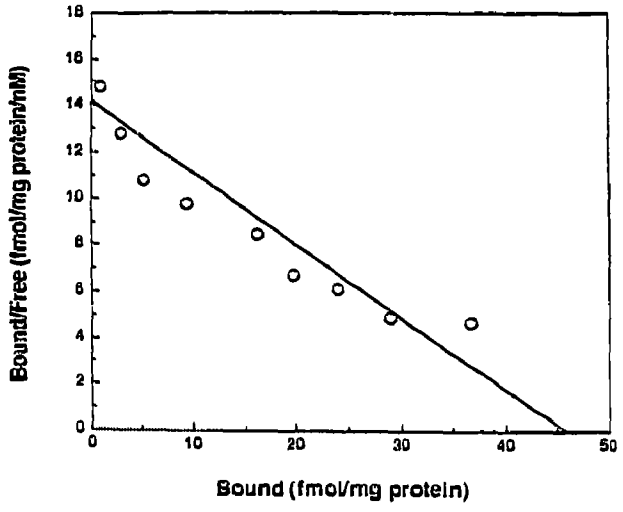

Fig. 1. Scatchard analysis of [^{125}I]PLA_2-I to crude membranes of guinea pig lung. The membranes were incubated in the presence of increasing concentrations of [^{125}I]PLA_2-I at 4°C for 2 h. Non-specific binding in the presence of 500 nM of porcine PLA_2-I was subtracted from each point. The specific binding was 85% of the total binding.

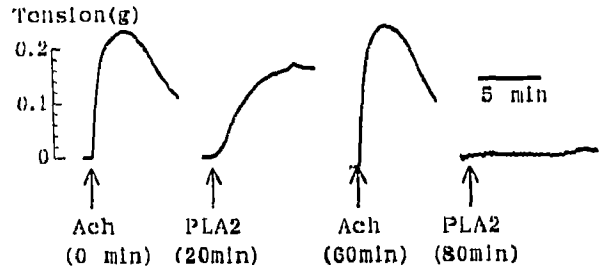

Fig. 2. Typical tracings showing the contractile response of guinea pig lung parenchymal strip to ACh and porcine PLA_2-I. After maximal contraction induced by 1 mM ACh (added at $t = 0$) was obtained, the tissue was washed with Kreb's solution and treated with 10 nM PLA_2-I at $t = 20$ min. After a further 20 min the tissue was washed and then 1 mM ACh and 10 nM PLA_2-I were subsequently added to the organ bath again at the indicated time.

was completely inhibited by porcine, human and rat PLA_2-I (100 nM), but was not affected by PLA_2-II's purified from rat and rabbit platelets and rat and human PLA_2-I zymogens (data not shown), indicating that guinea pig lung has a specific binding site for the mature form of mammalian PLA_2-I, as is the case for several rat preparations [3,4]. As shown in Fig. 2 porcine PLA_2-I (10 nM) elicited a slow contraction of guinea pig lung parenchymal strips. The contraction reached a plateau 5–10 min after the addition of PLA_2-I, and then declined slowly. After washing with Kreb's solution and returning to the baseline level the same amount of PLA_2-I was again added but no further contraction occurred, although the response to ACh remained unchanged. As can be seen in Fig. 3, the EC_{50} value (the PLA_2-I concentration giving a half-maximal response) was about 4 nM, a value which is similar to the K_d value (3.3 nM) for PLA_2-I binding. The maximal

Table I

Pharmacological modulation of porcine PLA_2-I-induced contractile response of guinea pig lung parenchyma

Drugs	Concentration (M)	*n*	Contraction (% of ACh)
Vehicle		12	61.3 ± 4.8
(+)-S-145Na	1×10^{-9}	4	28.2 ± 4.0**
	3×10^{-9}	4	17.4 ± 3.0**
	1×10^{-8}	6	5.5 ± 1.7**
ONO3708	1×10^{-7}	3	23.9 ± 3.3**
FPL55712	1×10^{-5}	8	61.7 ± 4.5
Pyrilamine	1×10^{-6}	4	70.9 ± 5.9
Indomethacin	1×10^{-5}	3	14.8 ± 2.0**

Lung parenchymal strips were pre-incubated with (+)-S-145Na, ONO3708, FPL55712, pyrilamine or indomethacin for 30 min, 30 min, 20 min, 5 min and 30 min, respectively. Then tissues were challenged consecutively with 10 nM porcine PLA_2-I. The contractile responses are expressed as a percentage of maximal response induced by 1 mM ACh. Each value represents the mean ± S.E.M. of the indicated number (*n*) of experiments. **Statistical significant from vehicle at $P < 0.01$.

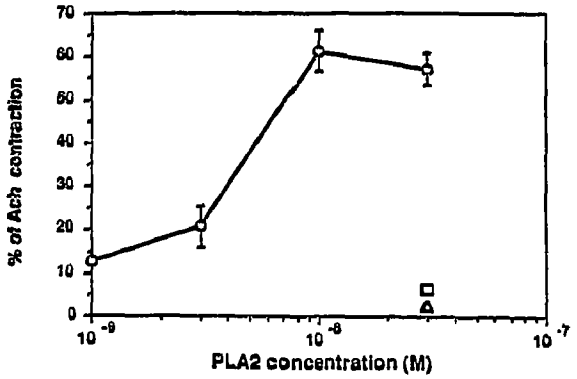

Fig. 3. Concentration–response curve for contractile responses of guinea pig lung parenchyma to porcine PLA_2-I (○), rat PLA_2-II (△), and the zymogen of porcine PLA_2-I (□). To minimize inter-tissue variability the contractile responses are expressed as a percentage of the maximal response induced by 1 mM ACh. Each point represents the means ± S.E.M., $n = 4$.

response to PLA_2-I was 50–70% of that to ACh. Neither porcine PLA_2-I zymogen nor rat PLA_2-II at 30 nM induced contraction significantly.

When lung parenchymal strips were pretreated with the TxA_2 receptor antagonist (+)-S-145Na or ONO3708, or the cyclooxygenase inhibitor indomethacin, the maximal contractile response induced by 10 nM PLA_2-I was markedly attenuated (Table I). In contrast no significant inhibition was observed in the presence of the LT antagonist FPL55712 or the histamine antagonist pyrilamine. It is, therefore, highly likely that PLA_2-I-induced contraction is mediated by TxA_2 produced by lung tissue in response to PLA_2-I.

In fact a marked increase in TxB_2 (stable metabolite of TxA_2) release from chopped lung tissue was detected 5 min after the addition of 10 nM PLA_2-I when marked contraction of lung strips was elicited. The treatment of

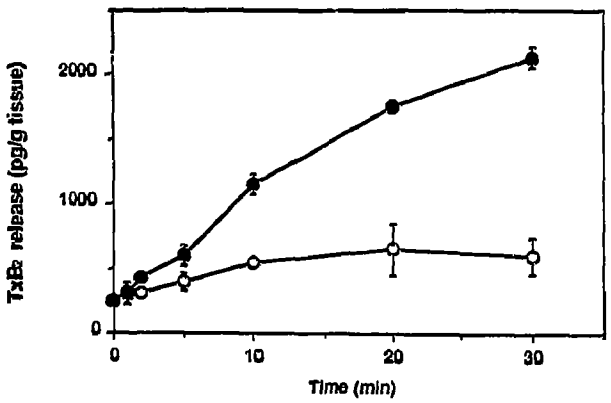

Fig. 4. Time–course of TxA_2 release from chopped guinea pig lung tissues by porcine PLA_2-I. The lung fragments were pre-incubated for 5 min and then challenged with vehicle (○) or 10 nM PLA_2-I (●). After an appropriate incubation period a portion was removed for determination of TxB_2 (see section 2). Each point represents the mean ± S.E.M., $n = 4$.

lung tissue with 10 nM PLA_2-I for 5 min also caused a significant release of $LTC_4/D_4/E_4$ (control, 68.1 ± 2.2; PLA_2-I treatment, 111.6 ± 10.0: $n = 4$, $P < 0.05$), but did not affect the release of PGD_2 and histamine. In view of the pharmacological evidence obtained with the LT receptor antagonist it is likely that the LT's released do not exert any direct effect on the contraction.

4. DISCUSSION

The present study indicates that PLA_2-I exerts a potent contractile effect in guinea pig lung parenchyma. This contraction was significantly attenuated by TxA_2 receptor antagonists and cyclooxygenase inhibitor but not by H1 blocker and/or LT receptor antagonist. These data, along with direct evidence of TxA_2 release from lung tissue, clearly indicate that TxA_2 may play a major role in the parenchymal contraction by PLA_2-I.

Guinea pig lung was found to have specific binding sites for the mature type of mammalian PLA_2-I and its K_d value was almost the same with an EC_{50} value for PLA_2-I-induced contraction. Rat PLA_2-II and zymogen of porcine PLA_2-I, which did not bind to the PLA_2-I binding site, could not induce the contraction of lung parenchyma at concentrations up to 30 nM. In addition treatment of the lung parenchyma by PLA_2-I (10 nM) followed by subsequent washing did not produce a response to the second challenge of PLA_2-I (Fig. 2), whereas the responses by other types of agonists, such as histamine, ACh and TxA_2 mimetic U46619, were not affected. This homologous desensitization, as well as type specific action of PLA_2's, strongly suggests that PLA_2-I-induced contraction is mediated via its specific receptor. Although the desensitization mechanism by PLA_2-I remains obscure one possible explanation is that it may be due to the rapid internalization of binding sites as has been found with cultured rat vascular smooth muscle cells [3]. PLA_2-I initially binds to its specific binding site in some target cells which may activate the arachidonic acid cascade within the cells to release TxA_2. As one of the possible candidates for the target cells, alveolar macrophages, which are mostly rich in TxA_2 synthase in lung [14], have been isolated from guinea pig, and examined for the effect of porcine PLA_2-I. However, no PLA_2-I binding site was found and no TxA_2 release was detected even when the cells had been treated with 100 nM porcine PLA_2-I for 30 min. Furthermore, no liberation of free fatty acids from alveolar macrophages labeled with [^{3}H]oleic acid or [^{3}H]arachidonic acid was detected up to 1 μM concentration of porcine PLA_2-I within 1 h, although 10 μM A23187 liberated a large amount of labeled fatty acids (data not shown).

Mast cells in lung parenchyma may not be involved in this activation process because histamine and PGD_2 were not significantly released in the initial response to PLA_2-I. Therefore, other types of pulmonary cells, i.e.

fibroblast, endothelial or smooth muscle cells might be involved as the target cells of PLA_2-I.

In conclusion, our findings reveal a new aspect of PLA_2-I action in lung as a possible mediator causing broncho-constriction in pulmonary diseases such as asthma [15]. We are now conducting further studies into its pathophysiological functions, as well as its target cells in lung tissue.

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