Enhanced expression of group II phospholipase A_2 gene in the tissues of endotoxin shock rats and its suppression by glucocorticoid

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We studied the regulation of group II phospholipase A_2 (PLA_2-II) gene in vivo, using endotoxin shock rat as a model for systemic inflammation. Administration of endotoxin into rats increased PLA_2 activity in the plasma, as described by Vadas and Hay, using endotoxin-challenged rabbit. Specific absorption of this activity by anti-PLA_2-II antibody indicated that the released PLA_2 was PLA_2-II. The levels of PLA_2-II mRNA were elevated in the aorta, spleen, lung, and thymus but not in the liver and kidney. The tissues with high PLA_2-II mRNA contents released a greater amount of PLA_2-II than the tissues of control rats. These results suggest that in endotoxin shock rats, PLA_2-II is synthesized de novo in the above tissues and released into circulation. Furthermore, our present study demonstrates that glucocorticoid suppresses the enhanced expression of the PLA_2-II gene in the tissues of endotoxin shock rats.

Phospholipase A_2; Endotoxin shock; Inflammation; Phospholipase A_2 mRNA; Glucocorticoid

1. INTRODUCTION

Several lines of evidence have suggested that secretory phospholipase A_2 (PLA_2) plays an important role in the pathogenesis of inflammatory diseases (for review see [2]). In the serum of Gram-negative septic-shock patients and experimental endotoxin-shock animals, levels of PLA_2 activity were significantly elevated [1,3,4]. Furthermore, intravenous infusion of PLA_2 into animals caused symptoms of septic shock [1,5,6]. Based on these findings, Vadas et al. have proposed that extracellular PLA_2 is a primary mediator of septic shock and endotoxin shock [7]. However, the type of circulating PLA_2, the source of the PLA_2, and the mechanism of the elevation of the PLA_2 levels are undefined as yet.

Two types of mammalian secretory PLA_2, termed group I and II, have been well characterized on the molecular basis. Group I PLA_2 is secreted from the cells as an inactive pro-enzyme, but group II PLA_2 (PLA_2-II) as an active enzyme [8,9]. We have recently clarified that some inflammatory factors increased PLA_2-II mRNA levels, which resulted in enhanced secretion of PLA_2-II from cultured rat smooth muscle cells [10,11]. This finding strongly suggests that PLA_2-II, which is often found in the extracellular fluid of some inflamed sites [12-14], participates in the progress of inflammatory processes. In this context, we thought that PLA_2-II might also be involved in endotoxin shock. In this study, we examined the activation of PLA_2-II gene in several tissues of endotoxin-challenged rats and investigated the effect of glucocorticoid on the activation.

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Abbreviations: PLA_2, phospholipase A_2; PLA_2-II, group II phospholipase A_2

2. MATERIALS AND METHODS

2.1. *Administration of endotoxin*

Male Sprague-Dawley rats weighing 280-320 g were used for all studies. Rats were injected intravenously with 5 mg/kg endotoxin (*Escherichia coli*, 055:B5, Sigma) dissolved in saline. Control rats received sterile saline. If necessary, rats were injected intraperitoneally with 10 mg/kg dexamethasone (Sigma) suspended in saline 30 min prior to administration of endotoxin. At timed intervals, blood obtained by puncture of the abdominal aorta was collected into plastic syringes containing 10 mM EDTA and 1 μg/ml prostaglandin E_1 to prevent blood coagulation and platelet activation. The blood was centrifuged (400 × *g*) at 4°C to obtain plasma. Tissues were immediately frozen with solid CO_2 just after extraction.

2.2. *PLA_2 activity assay*

PLA_2 activity was measured using [^{3}H]oleic acid-labeled (Amersham Corp.) *Escherichia coli* phospholipids as substrates [15]. The assay conditions are described elsewhere [10,11].

2.3. *Inhibition of PLA_2 activity by antibody*

Rabbit anti-rat PLA_2-II antibody was produced as described previously [10,11]. Samples were incubated with several dilutions of the antibody (7.3 mg IgG/ml) for 1 h, and PLA_2 activity was assayed. Rat pancreatic PLA_2 was purified as described by Ono et al [16].

2.4. *Release of PLA_2 from tissues*

About 0.2 g of aorta and thymus from control or endotoxin-treated rats was minced and incubated for 30 min in 2 ml of Dulbecco's modified Eagle's medium containing 0.1 mg/ml bovine serum

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Table I

Plasma PLA_2 activity in endotoxin-treated rats

Time (h)	PLA_2 activity (dpm)
0	401 ± 87
2	758 ± 123
4	1135 ± 297
8	2243 ± 45

Data are the mean ± SD ($n = 3$).

albumin. At the end of the incubation, the medium was collected and assayed for PLA_2 activity.

2.5. *RNA blotting*

Total RNA was prepared from the tissues by the method of Chomoczynski and Sacchi [17]. The RNA (10 μg) was fractionated on a 1% agarose gel containing 2.2 M formaldehyde. Hybridization of the RNA transferred to a nylon membrane was performed as described by Church and Gilbert [18], using rat PLA_2-II cDNA [9] labeled with [α-^{32}P]dCTP (Du Pont-New England Nuclear, 5000 Ci/mmol) as a probe.

3. RESULTS

As illustrated in Table I, PLA_2 activity in plasma was elevated in endotoxin-shock rats, which was induced by i.v. challenge with 5 mg/kg of *E. coli* endotoxin. In order to clarify the type of the plasma PLA_2, we carried out an inhibition study of the PLA_2 activity with a specific polyclonal antibody against rat PLA_2-II [10,11]. Fig. 1 shows that the antibody specifically inhibited the plasma PLA_2 activity but did not affect the activity of rat pancreatic PLA_2 which belongs to group I PLA_2. These results indicated that the PLA_2 whose levels in plasma were elevated by the endotoxin challenge was PLA_2-II.

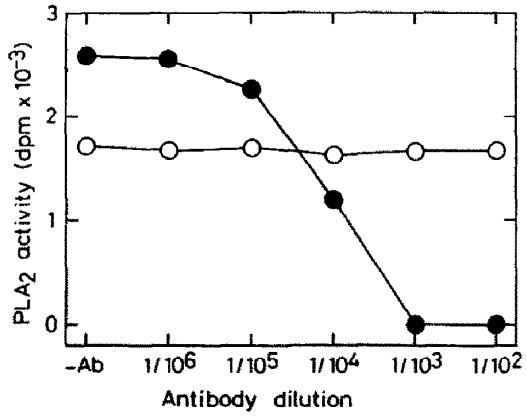

Fig. 1. Inhibition of plasma PLA_2 by anti-PLA_2-II antibody. Blood plasma collected 8 h after endotoxin challenge (•) or rat pancreatic PLA_2 (○) was incubated for 1 h at 25°C with several dilutions of anti-PLA_2-II antibody, and assayed for PLA_2 activity.

In previous studies [10,11], we have suggested that vascular smooth muscle cells are major PLA_2-II producing cells. Therefore, we next studied PLA_2-II mRNA levels in aorta of endotoxin-administered rats (Fig. 2). In the aorta of control rats, we hardly detected PLA_2-II mRNA by RNA blotting analysis. The mRNA

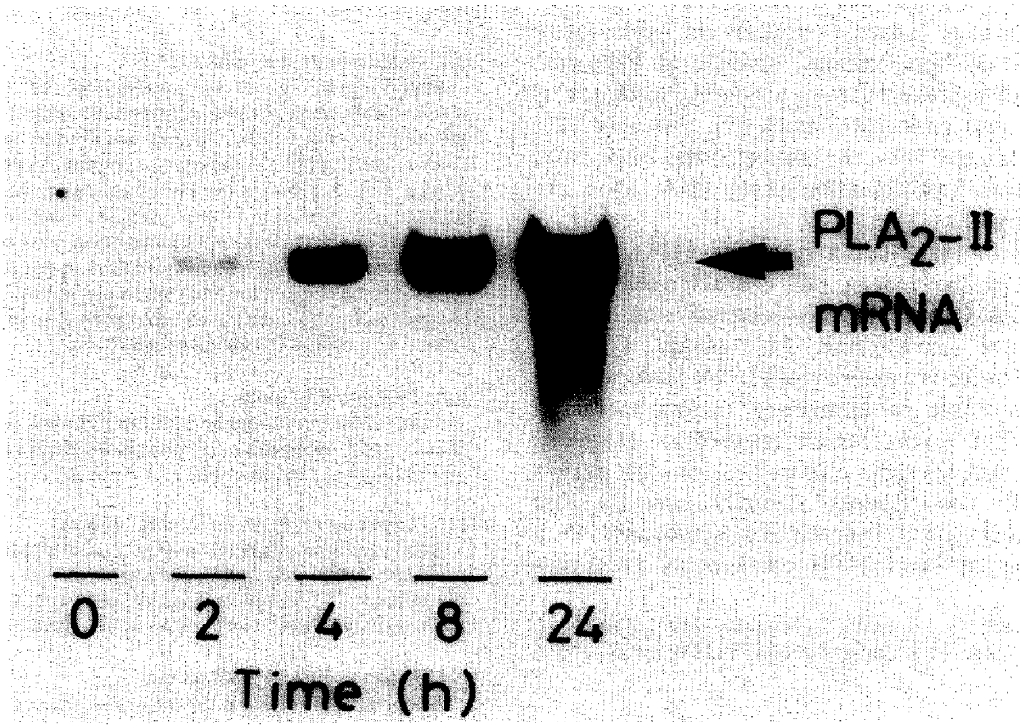

Fig. 2. Increase of PLA_2-II mRNA in aorta of endotoxin-challenged rats. Total RNA was prepared from aorta of control rats or endotoxin-administered rats. The RNA (10 μg) was analyzed by RNA blotting analysis as described in section 2.

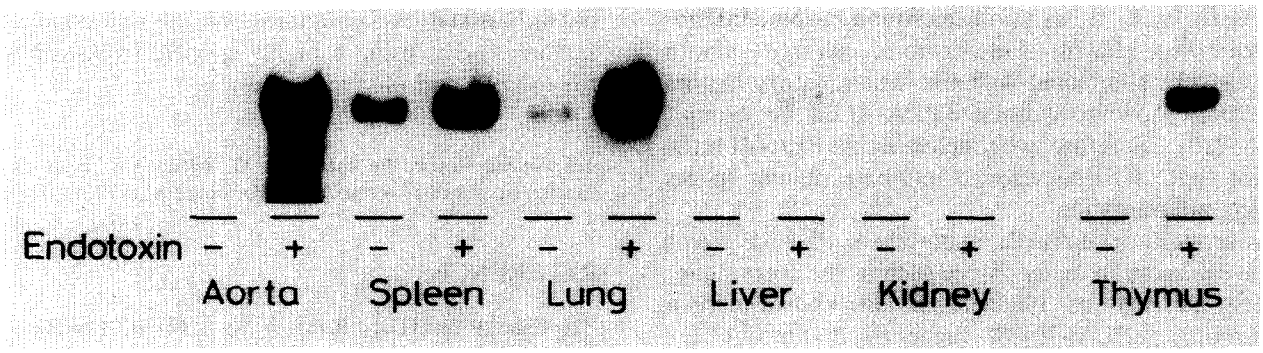

Fig. 3. Increase of PLA_2-II mRNA in tissues of endotoxin-treated rats. The tissues indicated in the figure were obtained from control rats or endotoxin-treated rats 24 h after administration of endotoxin. The RNA (10 μg) prepared from the tissues was analyzed as described in the legend of Fig. 2.

levels in aorta were markedly elevated upon administration of endotoxin. Furthermore, PLA_2-II mRNA levels also increased in the spleen, lung, and thymus by the endotoxin administration as shown in Fig. 3. However, PLA_2-II mRNA did not significantly increase in liver and kidney.

In order to further confirm the elevated synthesis of PLA_2-II protein by the tissues, PLA_2 activity released from the tissues during 30 min incubation was measured. As illustrated in Table II, the aorta and the thymus of endotoxin-challenged rats secreted greater amounts of PLA_2 activity than the tissues of control rats. The PLA_2 activity released from the tissues was completely inhibited by the anti-PLA_2-II antibody (data not shown), indicating that the PLA_2 released from the tissues was PLA_2-II. These findings verified that elevated mRNA levels in the tissues resulted in increased secretion of PLA_2-II into extracellular spaces.

Vadas and Hay [1] have previously demonstrated that glucocorticoids inhibited a rise in plasma PLA_2 activity in endotoxin-administered rabbits. On the other hand, we have recently reported that glucocorticoids inhibit the synthesis of PLA_2-II in cultured smooth muscle cells [11]. We thereby studied the effect of pretreatment of rats with dexamethasone, a synthetic glucocorticoid, on the increase of PLA_2-II mRNA in tissues evoked by endotoxin. Fig. 4 shows that the pretreatment of rats with 10 mg/kg dexamethasone alone had no effect on the mRNA levels, but it severely depressed the elevation of PLA_2-II mRNA levels by endotoxin in aorta, lung, and thymus.

Table II

Release of PLA_2 from tissues of endotoxin-treated rats

Tissue	PLA_2 activity (dpm)	
	Control	Endotoxin-treated
Aorta	729 ± 45	3477 ± 561
Thymus	1414 ± 686	3583 ± 788

PLA_2 activity released during 30 min incubation from tissues of control rats or endotoxin-treated rats was measured as described in section 2. Data are the mean ± SD ($n=3$).

4. DISCUSSION

In previous papers [10,11], we have shown that some inflammatory factors including endotoxin, tumor necrosis factor, and interleukin-1 stimulated accumulation of PLA_2-II mRNA in cultured rat vascular smooth muscle cells and enhanced secretion of PLA_2-II from the cells. In the present study, using endotoxin-administered rat as a model for systemic inflammation, we demonstrated for the first time that those responses observed in the cultured cells also occurred during in vivo inflammation.

Inhibition studies with anti-PLA_2-II antibody indicate that the PLA_2 activity in the endotoxin shock plasma and the PLA_2 activity released from the tissues of endotoxin-challenged rats are attributable to

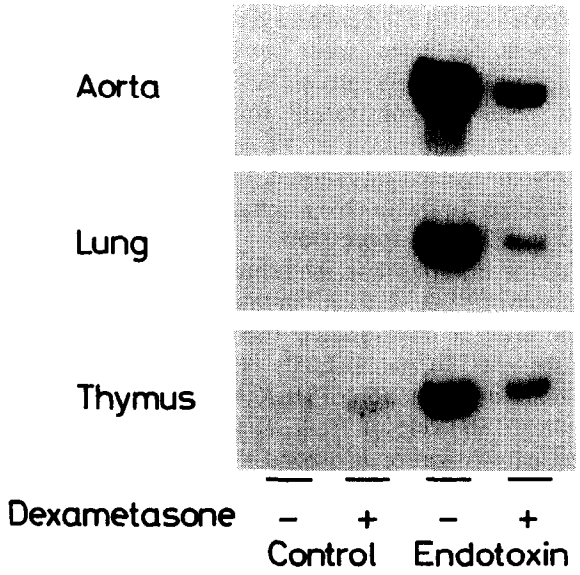

Fig. 4. Effect of dexamethasone on the increase of PLA_2-II mRNA in vivo. Rats were injected intraperitoneally with dexamethasone or saline, and then injected intravenously with endotoxin or saline 30 min after administration of dexamethasone. Tissues were removed and frozen 24 h after endotoxin challenge. The RNA (10 μg) prepared from the tissues was analyzed as described in the legend of Fig. 2.

PLA_2-II. PLA_2-II has also been found in some inflammatory sites [12–14]. Furthermore, cultured smooth muscle cells stimulated with the inflammatory factors have been shown to synthesize PLA_2-II but not group I PLA_2 [10]. Therefore, we concluded that PLA_2-II is the major type of PLA_2 secreted into extracellular spaces during inflammation.

In septic-shock animals, synthesis of PLA_2-II seems to be enhanced in many tissues such as the aorta, lung, spleen, and thymus. Platelets are also known to be a rich source of PLA_2-II [19], suggesting that most of the PLA_2-II in septic-shock serum is the enzyme released from platelets. However, during septic shock, the number of platelets decreases abruptly and severely [20]. Moreover, Murakami et al. [21] have demonstrated that activation of platelets in vivo induces a rapid and transient increase of plasma PLA_2 activity. Therefore, the long-term increase of plasma PLA_2-II as shown in Table I may not be due to the PLA_2-II secreted from platelets. PLA_2-II in endotoxin shock plasma may thus consist of de novo synthesized enzyme released from the above tissues.

Another important finding of this study is the fact that accumulation of PLA_2-II mRNA in the tissues of endotoxin-treated rats is suppressed by glucocorticoid. Glucocorticoid inhibits a rise in plasma PLA_2 activity in endotoxin-administered animals [1]. Thus far, inhibitory activities of glucocorticoids on PLA_2 action have been attributed to enhanced production of PLA_2-inhibitory proteins such as lipocortins by the agents [22,23]. Thus, one possible explanation of the glucocorticoid-mediated decrease of the PLA_2 activity in endotoxin-administered animals may be that the decrease is due to the enhanced secretion of lipocortins. However, our recent study has clarified that glucocorticoid inhibits the synthesis of PLA_2-II in vitro [11]. This result demonstrating that glucocorticoid suppresses the expression of PLA_2-II gene in tissues of endotoxin-treated rats should lead to new aspects to be considered for the in vivo mechanism of glucocorticoid-mediated PLA_2 suppression.

All the findings described thus far suggest an important role of PLA_2-II in inflammatory responses. In fact, some studies have shown the pro-inflammatory activities of PLA_2 [24,25]. However, biochemical evidence on the action of PLA_2 is still lacking. In order to clarify the role of PLA_2-II in inflammation, more precise investigation is needed of the PLA_2-II attacking sites as well as the correlation with the production of pro-inflammatory eicosanoids. We are now conducting further studies using a highly specific PLA_2 inhibitor for in vitro and in vivo studies.

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