Volume 309, number 3, 261–264 FEBS 11513 September 1992

Effect of pancreatic type phospholipase A_2 on isolated porcine cerebral arteries via its specific binding sites

Masatoshi Nakajima, Kohji Hanasaki, Motohiko Ueda and Hitoshi Arita

Shionogi Research Laboratories, Shionogi & Co., Fukushima-ku, Osaka 553, Japan

Received 6 July 1992; revised version received 22 July 1992

The addition of porcine pancreatic group I phospholipase A_2 (PLA_2-I) produced a transient contraction followed by a relaxation in helical strips of porcine cerebral arteries. Its ED_{50} value (2.3 nM) was almost identical to the K_d value (3.9 nM) calculated from the specific binding of ^{125}I-labeled porcine PLA_2-I in cultured porcine cerebral arterial smooth muscle cells. Type-specific action of PLA_2s and homologous desensitization strongly implicated the involvement of PLA_2-I-specific sites in the response. The transient contraction was abolished by treatment with indomethacin as well as by the removal of endothelium, indicating the dependence of vasoconstrictor prostoglandins synthesized by PLA_2-I in endothelium. The PLA_2-I-induced relaxation response was also observed in bovine and cat cerebral arteries, thus providing a new aspect of PLA_2-I as a vasoactive substance.

Phospholipase A_2; Specific binding site; Relaxation; Contraction; Porcine cerebral artery

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 act as a digestive enzyme in pancreatic juice [2]. However, in the membranes of a variety of cells and tissues, including those of human origin, we found a specific binding protein that recognizes a mammalian mature type of PLA_2-I [3, 4]. More recently, the PLA_2-I binding protein was purified from bovine corpus luteum membranes, and found to be composed of a single glycoprotein of M_r 190,000 [5]. PLA_2-I directly stimulated the DNA synthesis in Swiss 3T3 cells [3], rat synovial cells [4], rat vascular smooth muscle cells [4] and rat chondrocytes [6], and also induced chemokinetic migration in rat embryonic aortic smooth muscle cells (A7r5) [7] via its specific binding sites. Furthermore, binding of PLA_2-I to the specific sites resulted in a contraction of guinea pig lung parenchyma through the secondarily produced thromboxane A_2 [8]. These findings strongly implicate PLA_2-I in the modulation of cellular functions with respect to some pathophysiological states. In this study we characterized the PLA_2-I-specific binding sites in porcine cerebral arterial cells and examined the effect of PLA_2-I on mammalian cerebral arteries.

Correspondence address: H. Arita, Shionogi Research Laboratories, Shionogi & Co., 12-4 Sagisu 5-chome, Fukushima-ku, Osaka 553, Japan.

2. MATERIALS AND METHODS

2.1. *Materials*

Preparation of a variety of PLA_2s and iodination of porcine PLA_2-I were carried out as described in the previous paper [3, 4]. Drugs used were prostoglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (Ono Pharmaceutical Co., Osaka); papaverine hydrochloride (Dainippon Pharmaceutical Co., Osaka); nitroglycerin (Nihon Kayaku Co., Tokyo); substanace P (Peptide Institute, Minoh); cimetidine (Fujisawa Pharmaceutical Co., Osaka); (+)-chlorphenilamine maleate (Schering Corp., Kenilworth, NJ); Methylene blue trihydrate (Nakarai Chemicals Ltd., Kyoto); AA861 (Takeda Chemical Industries Ltd., Osaka); and indomethacin, atropine, propranolol, glybenclamide, Nw-nitro-L-arginine, NG-monomethyl arginine (Sigma, St. Louis, MO). FPL 55712 and CV 6209 were synthesized in our laboratories.

2.2. *Cell culture and binding experiments*

The basilar cerebral arteries were isolated from porcine brain obtained from a local slaughterhouse. After removal of the endothelium by gentle rubbing with a cotton pellet, smooth muscle cells were isolated from medial explants by the method of Ross [9]. The resulting cells were grown in Dulbecco's modified Eagle's medium supplemented with 20% fetal bovine serum (Gibco), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37°C. Cells were passaged by harvesting with 0.125% trypsin/0.01% EDTA. After serial subculture, the cells harvested between the fifth and eighth passages were used in the binding experiments. The binding assay using [^{125}I]PLA_2-I as a radioligand was carried out according to the method previously described [4].

2.3. *Responses to PLA_2-I of isolated cerebral arteries*

Porcine and bovine brains were obtained from a local slaughterhouse. Cats (weighing 3.5–5.5 kg) were anesthetized with intraperitoneal injections of sodium pentobarbital (30 mg/kg) and sacrificed by bleeding from the common carotid arteries. The brain was removed rapidly and basilar, middle cerebral and anterior cerebral arteries were isolated. The arteries were helically cut into strips of approximately 20 mm long. Each specimen was vertically fixed between hooks in a muscle bath containing modified Ringer-Locke solution, which was maintained at 37°C and aerated with a mixture of 95% O_2 and 5%

CO_2. The hook anchoring the upper end of the strips was connected to the lever of a force-displacement transducer (Nihon-kohden Kogyo Co., Tokyo). The resting tension was adjusted to 1.5 g for porcine cerebral arteries, 2.0 g for bovine cerebral arteries and 0.5 g for cat cerebral arteries. Constituents of the solution were as follows: (mM): NaCl 120; KCl 5.4; $CaCl_2$ 2.2; $MgCl_2$ 1.0; $NaHCO_3$ 25.0; and dextrose 5.6. Before the start of experiments, all strips were allowed to equilibrate for 90–120 min in the bathing media, during which time the medium was replaced every 10–15 min. Isometric contractions and relaxations were recorded on an ink-writing oscillograph. The contractile response to 30 mM K^+ was first obtained, then the preparations were repeatedly washed with fresh solution, and equilibrated for 30–40 min. The concentration-response relationship for PLA_2-I, proPLA_2-I or PLA_2-II was obtained by adding each PLA_2 directly to the bathing media in cumulative concentrations. To test the response of PLA_2-I, proPLA_2-I or PLA_2-II and the relaxant effect of substance P or isoproterenol, the arterial strips were partially contracted with $PGF_{2\alpha}$ (10^{-7} to 3×10^{-7} M) or serotonin (10^{-6} to 10^{-5} M), as previously described [10]. At the end of each series of experiments, papaverine (10^{-4} M) was applied to attain the maximum relaxation, which was taken as 100% relaxation. Preparations had been treated for 30–40 min with blocking agents before PLA_2-I was added. In some preparations the endothelium was removed by gently rubbing the intimal surface with a cotton pellet, and the response of endothelium-denuded strips was compared with that of strips with intact endothelium obtained from the same animals. Removal of endothelium was confirmed by a marked suppression of relaxation induced by substance P (10^{-7} M), and histologically by the method reported by Abrol et al. [11].

2.4. *Statistics*

Results shown in the text and figures are expressed as mean values ± S.E.M. Statistical analyses were made using Student's paired- and unpaired *t*-test.

3. RESULTS AND DISCUSSION

When [^{125}I]PLA_2-I (porcine) was incubated with cultured smooth muscle cells of porcine cerebral arteries, it bound specifically in a saturable manner. The Scatchard plot of the [^{125}I]PLA_2-I specific binding revealed the presence of a single class of binding sites with an equilibrium binding constant (K_d) of 3.9 nM and a maximum binding capacity (B_{max}) of 40 fmol/10^6 cells (data not shown). This K_d value is similar to those reported for other cells and tissues that possess specific PLA_2-I binding sites [4]. The specific binding of [^{125}I]PLA_2-I to the cells was completely inhibited by 100 nM of porcine, human and rat PLA_2-I but was not

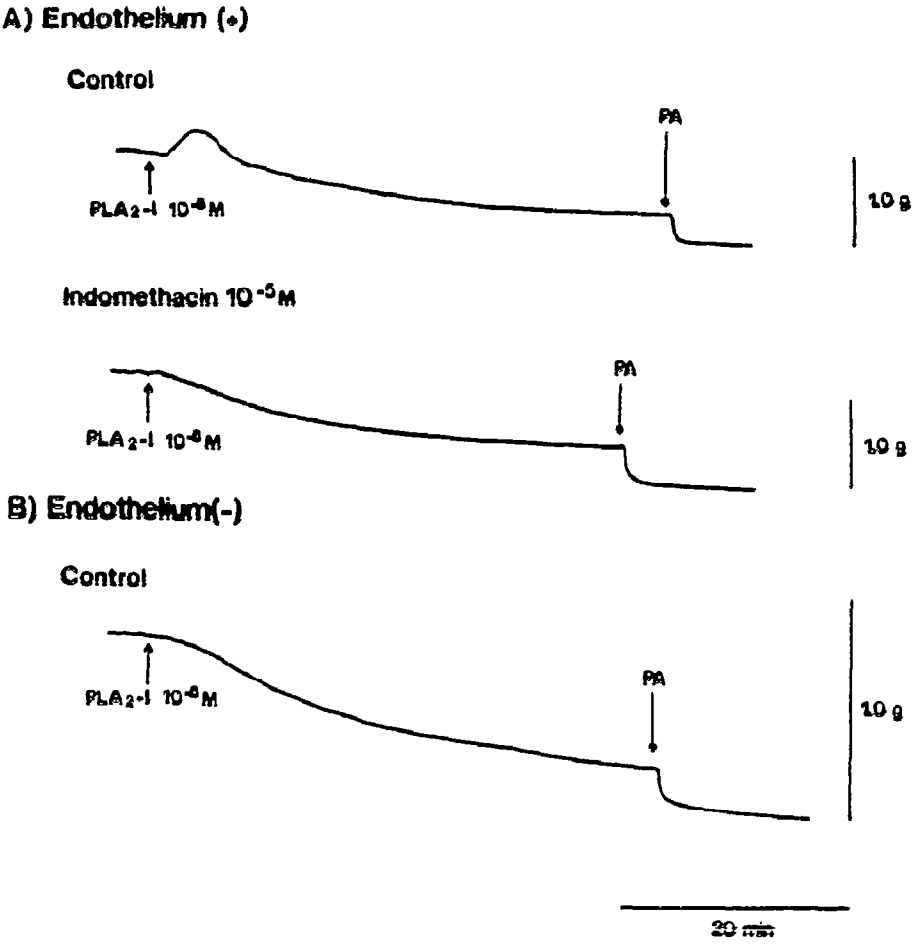

Fig. 1. Typical recordings of the responses to PLA_2-I (10^{-8} M) of porcine anterior cerebral arterial strips with (A) and without (B) endothelium or absence and presence of indomethacin (10^{-5} M). The strip was partially contracted with $PGF_{2\alpha}$ (10^{-7} M). The arterial strips were obtained from the same brain. PA (papaverine, 10^{-4} M) was then added to attain the maximum relaxation.

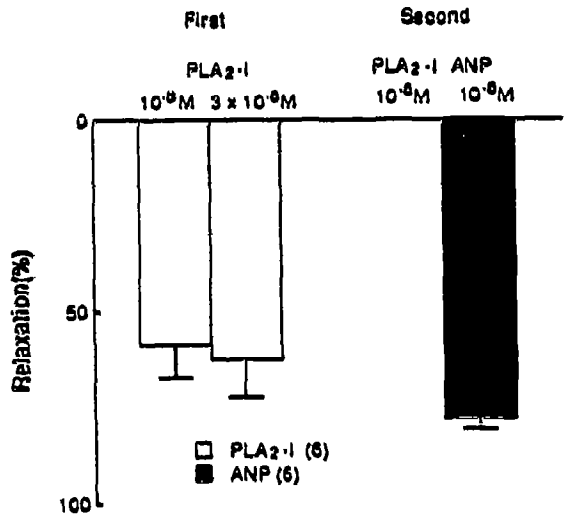

Fig. 2. Homologous desensitization of the PLA_2-I-induced relaxation in porcine anterior cerebral arteries partially contracted with $PGF_{2\alpha}$ (10^{-7} M) in the presence of indomethacin (10^{-6} M). After 30 min of the addition of PLA_2-I (10^{-8} M), a higher concentration of PLA_2-I (3×10^{-8} M) was added (First). The strip was then repeatedly washed with bathing media every 10 min to allow basal levels to be regained. After the strip was again pre-contracted with $PGF_{2\alpha}$ (10^{-7} M), PLA_2-I (10^{-8} M) or rat ANP (10^{-6} M) was added (Second). Relaxation induced by papaverine (10^{-4} M) was taken as 100%. Vertical bars represent S.E.M. and numbers in parentheses indicate the number of preparations examined.

affected by PLA_2-II purified from rat and rabbit platelets and the zymogens of rat and human PLA_2-I (data not shown), indicating that the cerebral arterial smooth muscle cells have a specific binding site for the mature type of mammalian PLA_2-I, as previously reported for several rat preparations [4–6]. Primary cultured porcine cerebral arterial endothelial cells were also confirmed to possess the PLA_2-I-specific binding sites in lesser amounts (K. Hanasaki and H. Arita, unpublished data).

As shown in Fig. 1, porcine PLA_2-I (10^{-8} M) elicited a transient contraction followed by a slowly developing relaxation in helical strips of cerebral arteries partially pre-contracted with $PGF_{2\alpha}$. The transient contraction was completely abolished by the treatment with indomethacin (10^{-5} M) or the removal of endothelium (Fig. 1), whereas the maximal relaxation was not significantly influenced (n=5); mean % relaxation in the strips with and without endothelium were 41.66 ± 4.06 (n=5) and 48.84 ± 2.27 (n=5), respectively, relative to relaxations caused by 10^{-4} M papaverine. These results suggest that PLA_2-I-induced transient constriction is mediated by some vasoconstrictive cyclooxygenase metabolites produced by the endothelial cells in response to PLA_2-I, whereas the relaxation response depends on the direct action of PLA_2-I in the smooth muscle cells. It has been reported that isolated cerebral arteries contract in response to cyclooxygenase products, such as $PGF_{2\alpha}$, PGE_2, PGD_2 and thromboxane A_2 [12, 13], which are

Table I

The relaxation response induced by PLA_2-I in porcine, bovine and cat cerebral arteries

	PLA_2-I-induced relaxation (%)		
	BA	MCA	ACA
Porcine	68.42 ± 5.16 (7)	45.94 ± 5.10 (7)	44.98 ± 4.43 (9)
Bovine	40.67 ± 10.59 (4)	53.36 ± 7.27 (4)	44.40 ± 7.41 (4)
Cat	43.83 ± 6.93 (7)	68.55 ± 8.33 (6)	

Each preparative strip was partially contracted with $PGF_{2\alpha}$ (10^{-7} M) in the presence of indomethacin (10^{-6} M), and then PLA_2-I (10^{-8} M) was added. The PLA_2-I-induced relaxation was expressed as a percentage of the response induced by papaverine (10^{-4} M). Numbers in parentheses indicate the number of preparations examined. BA, basilar artery; MCA, middle cerebral artery; ACA, anterior cerebral artery.

known to be synthesized in cerebral arterial endothelium [10]. Although vasodilator PGs, such as PGI_2, may also be released by PLA_2-I, this prostanoid (10^{-9} M to 10^{-6} M) could not induce relaxation of porcine cerebral arteries (M. Nakajima, unpublished data).

In the arteries treated with indomethacin, 10^{-8} M PLA_2-I caused only a slow developing relaxation (Fig. 1), however, further addition of PLA_2-I (3×10^{-8} M) resulted in no additional relaxation (Fig.2). After washing with the bathing media and returning to the baseline level, PLA_2-I (10^{-8} M) was again added. As shown in Fig. 2 (Second), no further relaxation occurred, although a response to rat atrial natriuretic polypeptide (ANP) (10^{-8} M) could be detected. The homologous desensitization mechanism by PLA_2-I may be explained by rapid internalization of the PLA_2-I binding site, as has been found in rat vascular smooth muscle cells [4]. As shown in Fig. 3, the EC_{50} value (the PLA_2-I concentration giving a half-maximal relaxation response) was 2.32 ± 0.16 nM (n=8), a value which is similar to the K_d value (3.9 nM) for PLA_2-I binding. On the other hand, neither porcine pro-PLA_2-I nor rat PLA_2-II induced relaxation significantly. The relaxations induced by PLA_2-I (10^{-8} M) were also observed in arteries partially contracted with serotonin; 51.8 ± 5.18% (n=7). As summarized in Table I, PLA_2-I (10^{-8} M) induced relaxation of basilar, middle cerebral and anterior cerebral arteries prepared from porcine, bovine or cat, whereas any significant effects could not be observed in the thoracic aortic strips.

The effects of several inhibitors or receptor antagonists on the PLA_2-I-induced relaxation were than examined in porcine cerebral arteries. The PLA_2-I-induced responses could not be influenced by 10^{-6} M atropine (muscarinic receptor antagonist), 10^{-6} M propranolol (β-receptor antagonist), 10^{-5} M cimetidine (H_2-receptor antagonist), 10^{-5} M chlorphenylamine (H_1-receptor antagonist), 10^{-5} M FPL 55712 (leukotriene antagonist), 10^{-5} M CV 6209 (PAF antagonist), 10^{-5} M AA861 (5-lipoxygenase inhibitor), 10^{-6} M glybenclamide (ATP-

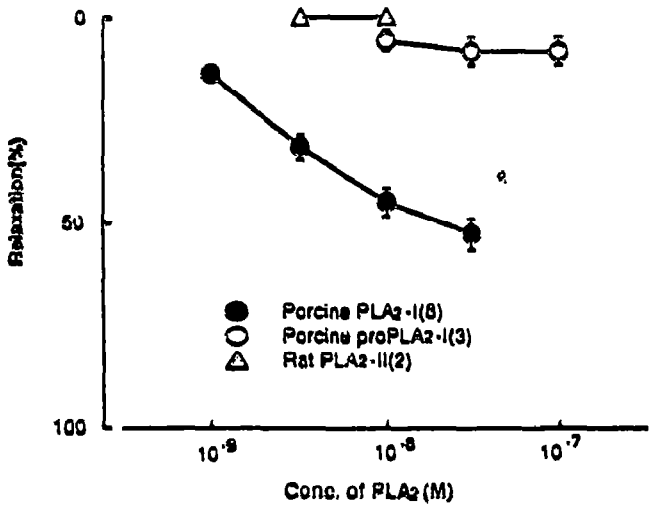

Fig. 3. Concentration–response curve for the relaxation of porcine anterior cerebral arteries in the presence of indomethacin (10^{-6} M) in response to porcine PLA_2-I, porcine proPLA_2-I and rat PLA_2-II. The strips were partially contracted with $PGF_{2\alpha}$. The maximum relaxation obtained by the addition of 10^{-4} M papaverine was taken as 100%. Vertical bars represent S.E.M. Numbers in parentheses indicate the number of preparations examined.

sensitive K channel blocker), 10^{-5} M Methylene blue (soluble guanylate cyclase inhibitor), 10^{-4} M Nw-nitro-L-arginine (NO synthase inhibitor) and 3×10^{-5} M NG-monomethyl arginine (NO synthase inhibitor). Treatment with PLA_2-I (10^{-8} M) did not affect the concentration–response curves of nitroglycerin (10^{-8} to 10^{-5} M) (n=4) and isoproterenol (10^{-8} to 10^{-5} M) (n=4). Furthermore, any significant increase in cGMP (guanosine 3',5' cyclic monophosphate) and cAMP (adenosine 3',5' monophosphate) levels could not be detected in cultured porcine cerebral arterial smooth muscle cells stimulated by PLA_2-I (10^{-7} M), whereas rat ANP (10^{-7} M) and PGE_1 (10^{-6} M) significantly enhanced the cGMP and cAMP levels, respectively (K. Hanasaki and H. Arita, unpublished data). Thus, unknown intracellular second messengers might be involved in the PLA_2-I-evoked relaxation via its specific receptor in cerebral arterial smooth muscles.

In conclusion, our findings reveal a new aspect of PLA_2-I action in cerebral arteries as a vasoactive substance. We are now conducting further studies into its pathophysiological functions, as well as signal transduction mechanisms under the PLA_2-I-evoked responses.

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