

Original Article

Immunohistochemical Localization of PhospholipaseA₂ in the Guinea Pig Nasal Mucosa

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Objective: Phospholipase A₂ (PLA₂) is a key enzyme in arachidonic acid metabolism, which is involved in the maintenance of biological homeostasis and the onset of various diseases. The immunohistochemical localization of PLA₂ in the nasal mucosa has not been reported, even though the presence of messenger RNA of PLA₂ has been demonstrated in the human nasal brush sample. The present study was designed to determine the localization of PLA₂s in the nasal cavity.

Methods: The immunohistochemical localization of secretory PLA₂ (sPLA₂) and cytosolic PLA₂ (cPLA₂) in the nasal mucosa was studied using adult guinea pig.

Results: Both sPLA₂ and cPLA₂ were localized in the nasal gland as well as the respiratory epithelium, and not in the surrounding vascular endothelial cells, olfactory gland, olfactory epithelium or submucosal tissue.

Conclusion: Our data provide the first convincing evidence that both sPLA₂ and cPLA₂ are significantly expressed in the nasal gland and the respiratory epithelium, and are suggested to regulate the function of the nasal mucosa, such as bactericidal, Na secretion, and allergic response.

Key words: phospholipaseA₂, arachidonic acid, nasal gland, immunohistochemistry

Introduction

Mechanical or chemical stimulation of the cell membrane by various agonists results in activation of PLA₂ on the membrane. Activated PLA₂ then triggers production of numerous lipid mediators, representing an important step in signal transduction. Arachidonic acid is one such lipid mediator that is subsequently converted into prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs) (collectively called eicosanoids) mediated by cyclooxygenase (COX) and 5-lipoxygenase (5-LOX), respectively. Thus, products of PLA₂ act as intracellular second messengers in signal transduction, implicating that PLA₂ is related to such regulation, thereby contributing to various physiological roles¹. The presence of messenger ribonucleic acids (mRNAs) for different PLA₂ forms has been demonstrated in the human nasal epithelial cells, collected by brush². Further, the increased PLA₂ activity in the nasal lavage fluid from allergic patients after allergen provocation has been demonstrated^{3,4}. However, histological localization of PLA₂ in the nasal mucosa has not been examined previously. The purpose of the present study was to clarify immunohistochemical localization of PLA₂ in the nasal mucosa.

MATERIALS and METHODS

Ten young adult female Hartley guinea pigs weighing about 250–300 g were studied. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University. Under general anesthesia with sodium pentobarbital (50mg/kg), animals were exsanguinated

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by transcardial perfusion with physiological saline containing 0.5% sodium nitrite and then 0.5% zinc-10% formalin solution (pH 5.0). The nose was then removed and immersed in the same fixative for another 2 hours. After decalcification with 5% ethylenediaminetetraacetic acid (pH 7.3) at 4°C for 2 weeks, the specimens were dehydrated with ethanol, embedded in paraffin and sectioned serially at 20 μ m in thickness. After deparaffinization, specimens were incubated overnight at 4°C with commercially available primary antibody against cPLA2, derived from human U937 cells (rabbit polyclonal antibody, N-216, catalogue number sc-438, Santa Cruz Biotechnology, Santa Cruz, CA), by which the specific localization of cPLA2 in the cerebellum of the adult macaque could be demonstrated by indirect immunofluorescence⁵. The specimens were also immunostained overnight at 4°C with antibody against type I sPLA2 derived from porcine pancreas (rabbit polyclonal antibody, catalog number #06-150, Upstate Biotechnology, Lake Placid, NY), which has been previously used in the immunolocalization study of the mouse cochlea⁶. Although this antibody can recognize other sPLA2s than type I sPLA2, we chose the above antibody because the aim of the present study is to demonstrate the localization of any form of sPLA2 in the nasal mucosa as in the previous study⁶. Both antibodies were diluted 100-fold with 0.01 M phosphate buffer solution (PBS, pH 7.3). The specimens were then treated with anti-rabbit IgG (Vector Laboratories, Burlingame, CA) and with ABC reagents (Vector Laboratories) for 60 minutes, respectively. The sites of bound primary antibodies were visualized by development in 3,3'-diaminobenzidine solution. In order to confirm reaction specificity, the immunohistochemical reaction was tested by replacing the primary antibody with nonimmune rabbit serum (negative control). The specificity of the two antibodies used in our study was investigated by performing immunohistochemical staining of the specimen which has been well known for cPLA2 and sPLA2 immunoreactivity^{7,8}. A section of kidney and gastric glands, known to express immunohistochemically cPLA2 and sPLA2, respectively was used as positive external tissue control for cPLA2 and sPLA2 staining.

RESULTS

The PLA2s exhibited a cell specific expression pattern in the nasal mucosa of the young adult guinea pig.

Both cPLA2 and sPLA2 showed an intense immunohistochemical reaction in the nasal gland and less intense reaction in the respiratory epithelium (Fig. 1). No specific reaction was observed in the endothelial cells of surrounding vessels, olfactory gland, olfactory epithelium or submucosal tissues. Examination of the control sections revealed localization of cPLA2 in the proximal tubules in the kidney but not in the stomach (data not shown). Conversely, sPLA2 expression was observed in the chief cells of the gastric fundus but not in the kidney (data not shown). No immunoreactivity was observed in control sections exposed to nonimmune rabbit serum instead of the primary antibody. These results confirmed the specificity of the two antibodies, anti-cPLA2 and anti-sPLA2, that we used in this study.

DISCUSSION

The PLA2 superfamily is divided into several groups based on their molecular weight, structure, cellular localization, and Ca^{2+} requirements⁹, although these isozymes perform the same catalytic function. In particular, two main isoforms of mammalian Ca^{2+} -dependent PLA2, namely cPLA2^{10,11}, which is present in the cytosol of a variety of cell types, and sPLA2¹², which generally contains a secretory signal peptide and is localized in cellular granules of various organs, have been cloned and sequenced. PLA2 is thought to participate in the intracellular signal transduction processes in many organs involved in the production of PGs and TXs¹. In the nose PG-D2, which is the most abundant arachidonic acid metabolite¹³, is known to induce swelling of the nasal mucosa¹⁴ and enhance mucin production¹⁵. Similarly, TX-A2, another PLA2-related arachidonic acid metabolite, causes dilatation of microvessels in the nose to induce interstitial edema, resulting in nasal mucosal congestion and increased nasal discharge¹⁶. Moreover, a large number of both cPLA2 and sPLA2 types have been reported to be present in the normal human nasal mucosa² and the mRNA levels of PLA2 VIIA (sPLA2, platelet-activating factor (PAF) acetylhydrolase) were lower in patients with seasonal allergic rhinitis than controls, regardless of whether there is ongoing allergic inflammation or not¹⁷. Taken together, it is evident that arachidonic acid metabolites induced by PLA2 and PLA2 itself display a variety of physiological function in the nose. However, histological localization of PLA2 has not been studied yet, even though mRNAs for PLA2 has been demon-

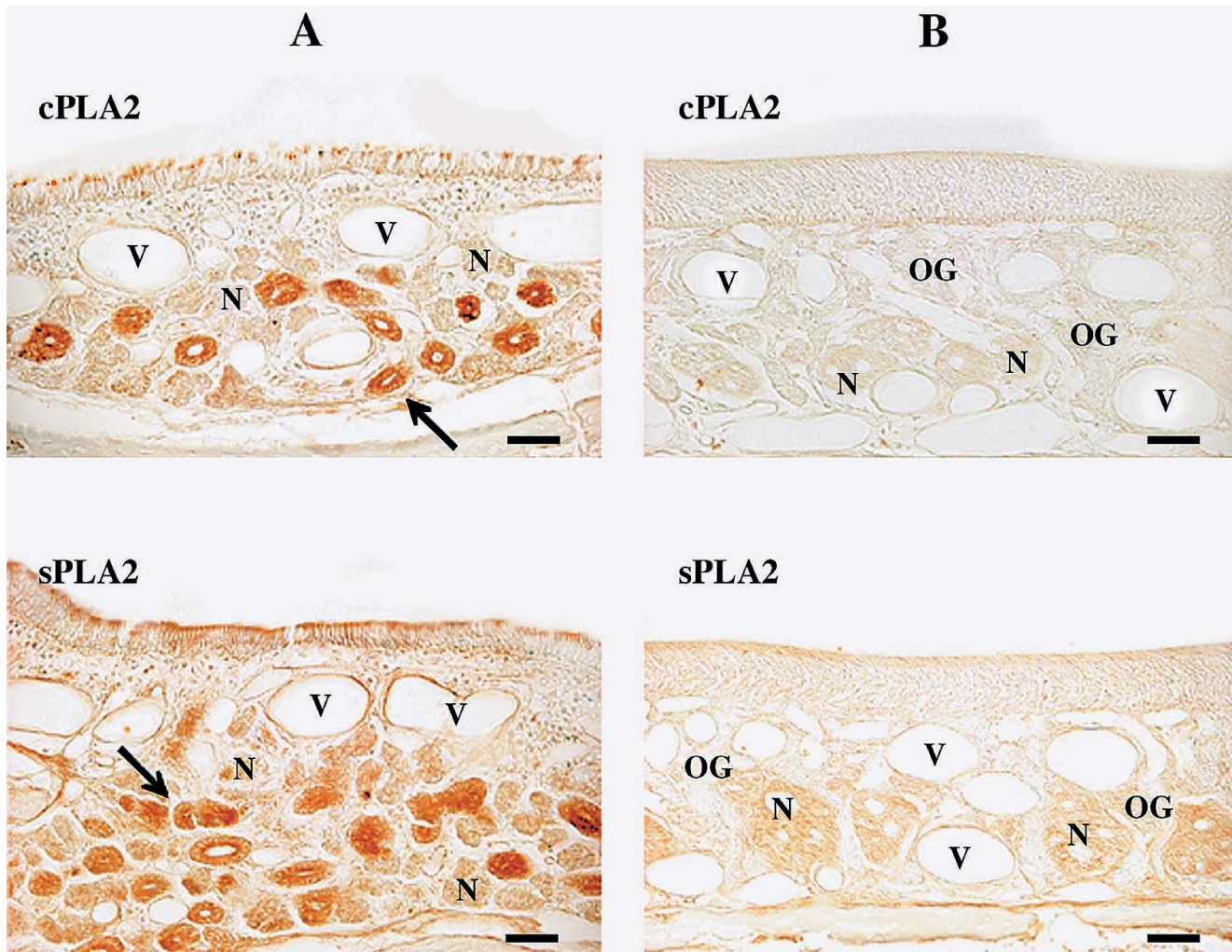


Fig. 1. Immunohistochemical localization of cPLA2 and sPLA2 in the nasal mucosa.

A) respiratory epithelium, B) olfactory epithelium. Both cPLA2 and sPLA2 were detected in the nasal glands (arrow) and in the respiratory epithelium, but not in the submucosal tissue, vascular (V) endothelial cells, or in nerves (N). Either cPLA2 or sPLA2 immunoreactivity was not detected in the olfactory epithelium, olfactory glands (OG), vascular (V) endothelial cells, or in nerves (N).

Bar = 50 μ m.

strated in the human nasal epithelial cells collected by brush². Thus the present study was designed to localize cPLA2 and sPLA2 in the guinea pig nose employing immunohistochemical techniques.

Our results confirmed the presence of both cPLA2 and sPLA2 in the nasal glands as well as the respiratory epithelium. The sample in which mRNA of the PLA2 was demonstrated, was obtained by nasal lavage or brushing². Nasal glands can hardly be collected by these method. Therefore, mRNA of the PLA2 demonstrated by nasal lavage or brushing were suggested to originate from the respiratory epithelium. sPLA2, expressed in the stomach, is known to be secreted into the lumen of the stomach⁸. Given that

sPLA2 is intensely immunostained in the nasal gland, sPLA2 might be secreted from nasal glands into the nasal mucous, in a similar mechanism to that in the chief cells of the stomach. In addition, sPLA2 is known to possess antibacterial properties²¹. The sPLA2 in the nasal mucous may serve to interrelate with the defense mechanism against bacterial infection.

Considering that cPLA2 is involved in the control of secretion of Na⁺ and water in the renal tubules¹⁸, cPLA2 expressed in the nasal gland is expected to be involved in the secretion of Na⁺ and water in the nasal gland. In support of our finding, COX, a downstream enzyme of PLA2 in the arachidonic acid cascade, was also detected in the nasal gland¹⁹. COX is also known

to be present in the rat renal collecting duct and to be involved in the Na-K-ATPase regulation and modulation of the pump indirectly by reducing luminal Na entry²⁰. This is consistent with our hypothesis that cPLA2 regulates Na⁺ and water secretion in the nasal gland.

sPLA2 has been reported to be secreted in response to inflammation and to act as an acute-phase protein²¹. The nasal mucosa is constantly exposed to environmental factors such as chemicals, allergens and microorganism and the presence of PLA2 may therefore reflect a need for a fast response and a first line of defense towards all these stimuli. The molecular mechanism by which PLA2 is involved in the nasal mucosal function remains to be fully elucidated. Further studies are required to clarify the function of PLA2.

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