Basic fibroblast growth factor stimulates cytosolic phospholipase A2, phospholipase C-γ1 and phospholipase D through distinguishable signaling mechanisms

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Abstract

Fibroblast growth factors (FGFs) stimulate proliferation, differentiation and motility of different cell types. The cellular effects of FGF are transduced by its interaction with any one of four members of a family of high affinity, cell surface FGF receptors (FGFRs) that have autophosphorylating tyrosine kinase activity. Activation of FGFR causes release of various low molecular weight signaling molecules which are required for the pleotropic effects of FGFs. We report here that basic FGF plays critical role in membrane phospholipid hydrolysis in NIH 3T3 cells that are stably transfected with FGFR1. Upon binding to FGFR1, basic FGF stimulates cytosolic form of phospholipase A2 (cPLA2), phospholipase C-γ1 (PLC-γ1) and phospholipase D (PLD), the key enzymes for the production of various lipid second messengers, in a tyrosine kinase-dependent manner. In addition to tyrosine phosphorylation, cPLA2 catalytic activation requires serine phosphorylation by p42 mitogen-activated protein (MAP) kinase and possibly pertussis toxin-sensitive G-protein coupling. On the other hand, phosphatidyl inositol 4,5 bisphosphate (PIP2) hydrolysis requires direct phosphorylation at tyrosine residue of the PLC-γ1 isozyme. The activation of PLD needs direct or indirect receptor tyrosine kinase and protein kinase C (PKC) activities. Additionally, it also requires botulinum toxin C-sensitive Rho-like G-protein activation. All these results suggest that the pleotropic effects of FGF are exerted through its tyrosine kinase receptors and individual effectors are activated via distinguishable signaling mechanisms according to the cell’s need.

Introduction

Basic fibroblast growth factor (FGF) is a potent mitogenic and chemotactic factor for a variety of cell types [1]. Its activity has been implicated in multiple physiological and pathological processes including differentiation, wound healing and tumor angiogenesis [2]. The pleotropic effects of FGF are transduced by its interaction with any one of four members of a family of high-affinity, cell surface FGF receptors. These receptors are ‘single pass’ transmembrane
proteins with a kinase activity that induces phosphorylation of tyrosine residues in the receptor itself, as well as other intracellular substrate proteins [3–5]. Basic FGF also triggers a series of downstream events including activation of p21ras [6], ras-like protein Rho [7], mitogen-activated protein (MAP) kinase [8, 9] and expression of early responsive genes [10]. In addition, basic FGF rapidly induces the release of a number of low molecular weight signaling molecules including arachidonate, inositol 1,4,5-trisphosphate (IP_{3}), diacylglycerol (DAG), phosphatidic acid (PtdOH) [11–13], etc. But the sequential mechanisms by which this pleotropic agent induces the release of these important biomolecules are not well established.

Eicosanoids derived from arachidonate elicit multiple physiological and pathophysiological responses. We have previously demonstrated that arachidonate release is necessary for FGF-stimulated endothelial cell motility [11], and that this important biomolecule is derived as a result of activation of cytosolic phospholipase A_{2} (cPLA_{2}) [9]. Various studies have shown that cPLA_{2} is activated by phosphorylation by MAP kinase [9, 14]. The phosphorylated enzyme is translocated from cytosol to membrane in a process utilizing Ca^{2+}-dependent phospholipid-binding domain [15].

As with other growth factors, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), etc., the binding of FGF to its receptors leads to phospholipase C-γ1 (PLC-γ1) activation by tyrosine phosphorylation [3]. Activated PLC-γ1 stimulates PIP_{2} hydrolysis, forming two second messengers, IP_{3} and DAG, which releases sequestered calcium from a subpopulation of the endoplasmic reticulum, and diacylglycerol (DAG). DAG and Ca^{2+} in turn activate protein kinase C (PKC) [16]. These two signaling molecules participate in the transduction of different mitogenic signals across the plasma membrane and regulate various cellular events. Mohammadi et al. [17] and Peters et al. [18] independently demonstrated that point mutation in the FGF receptor selectively eliminates activation of PLC-γ1 and that phosphatidylinositol (PtdIns) hydrolysis is not essentially required for FGF-induced mitogenesis.

Phosphatidylycercholine (PtdCho) constitutes the largest fraction of total plasma membrane phospholipids and was previously thought to be metabolically rather stable compared to PtdIns, However, PtdCho has been shown to be hydrolyzed in response to various extracellular signal molecules upon receptor-mediated activation of phospholipase A_{2}, C and D [19]. Recent evidences show that many agonists, including FGF, stimulate DAG production through the hydrolysis of PtdCho and that PLD is the major enzyme involved [13]. Phospholipase D initially produces PtdOH, which may have second messenger role, but can also be rapidly converted by PtdOH-phosphohydrolase to DAG [20]. Most agonists induce a biphasic production of DAG, with PtdIns-PLC being responsible for the initial rapid increase and PtdCho-hydrolyzing PLD for the second sustained increase. Phospholipase D-catalyzed PtdOH formation has been shown to inhibit G2/M phase transition of a number of cultured cells in a reversible manner as a part of their biomodal function in growth control [21]. Although agonist-induced activation of PLD is a widely occurring phenomenon, the regulatory mechanisms involved are not well defined. Evidence for the involvement of PKC in the regulation of PLD has been obtained in many different cell systems with various agonists. Besides PKC, receptor tyrosine kinases themselves, Ca^{2+}, and small GTP-binding protein (G-protein) rho has been shown to be involved in the regulation of cellular PLD activity. However, evidence for the sequential mechanisms is limited, and there have been several reports presenting contradictory findings [13].

We designed the present study to identify possible mechanisms by which basic FGF activates PLA_{2}, PLC-γ1 and PLD in NIH 3T3 cells stably expressing FGFR1. It is demonstrated that basal phospholipases in these cells are activated by various different mechanisms apparently involving tyrosine phosphorylation, PKC, MAP kinase and G-proteins. Evidence is provided that hydrolysis of phospholipids involved receptor tyrosine kinase as an initial step.

**Materials and methods**

Myelin basic protein (MBP), phorbol 12-myristate 13-acetate (PMA), 5'-methylthioadenosine (MTA) and general reagents for kinase assay were purchased from Sigma, USA. PKC assay kit, herbimycin A and fetal bovine serum (FBS), Dulbecco’s modified Eagle’s (DME) medium etc., were procured from Gibco BRL, USA. [γ-^{32}P]ATP (specific activity 3000 Ci/mmmole) was purchased from BRIT, India. Phosphatidyl [2-^{3}H]inositol 4,5-bisphosphate (1'H)PIP_{2} (specific activity 6 Ci/mmmole), [9,10(n)-^{125}I]oleic acid (10 Ci/mmol) and L-α-1-palmitoyl-2-[^{14}C]arachidonyl phosphatidylcholine (specific activity 53 mCi/mmol) and [125]protein A were purchased from Dupont NEN, USA. Pertussis toxin, cholera toxin and H7 were procured from Biomol inc. USA. Botulinum toxin C3 was obtained from Calbiochem, USA. Monoclonal anti-PLC-γ1 antibody and anti-phosphotyrosine antibodies (both monoclonal and polyclonal) were obtained from Santa-Cruz, USA. Rabbit polyclonal anti-cPLA_{2} was obtained as described previously [9]. Polyclonal antibody against p24 MAP kinase (TR110) [8] was kindly supplied by Dr. M Weber, University of Virginia, USA and sucrose monolaurate was a gift from Dr. M-J Im, Cleveland Clinic Foundation, USA.

**Cell culture**

NIH 3T3 cells overexpressing transfected human FGFR1 or control vector (obtained from Dr. M Jaye of Ronae Polancae...