Biochimica et Biophysica Acta 1813 (2011) 2145–2156

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

PKCθ activation in pancreatic acinar cells by gastrointestinal hormones/neurotransmitters and growth factors is needed for stimulation of numerous important cellular signaling cascades

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ARTICLE INFO

Article history:
Received 9 February 2011
Received in revised form 12 July 2011
Accepted 13 July 2011
Available online 23 July 2011

Keywords:
PKCθ activation
Pancreatic acini
CCK
Signaling
Pancreatic growth factors
PKC

ABSTRACT

The novel PKCθ isoform is highly expressed in T-cells, brain and skeletal muscle and originally thought to have a restricted distribution. It has been extensively studied in T-cells and shown to be important for apoptosis, T-cell activation and proliferation. Recent studies showed its presence in other tissues and importance in insulin signaling, lung surfactant secretion, intestinal barrier permeability, platelet and mast-cell functions. However, little information is available for PKCθ activation by gastrointestinal (GI) hormones/neurotransmitters and growth factors. In the present study we used rat pancreatic acinar cells to explore their ability to activate PKCθ and the possible interactions with important cellular mediators of their actions. Particular attention was paid to cholecystokinin (CCK), a physiological regulator of pancreatic function and important in pathological processes affecting acinar function, like pancreatitis. PKCθ-protein/mRNA was present in the pancreatic acini, and T538-PKCθ phosphorylation/activation was stimulated only by hormones/neurotransmitters activating phospholipase C. PKCθ was activated in time- and dose-related manner by CCK, mediated 30% by high-affinity CCK_A-receptor activation. CCK stimulated PKCθ translocation from cytosol to membrane. PKCθ inhibition (by pseudostrate-inhibitor or dominant negative) inhibited CCK- and TPA-stimulation of PKD, Src, RafC, PYK2, $p125^{FAK}$ and IKKα/β, but not basal/stimulated enzyme secretion. Also CCK- and TPA-induced PKCθ activation produced an increment in PKCθ's direct association with AKT, RafA, RafC and Lyn. These results show for the first time the PKCθ presence in pancreatic acinar cells, its activation by some GI hormones/neurotransmitters and involvement in important cell signaling pathways mediating physiological responses (enzyme secretion, proliferation, apoptosis, cytokine expression, and pathological responses like pancreatitis and cancer growth).

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1. Introduction

Protein kinase C θ (PKCθ) belongs to the threonine/serine kinase superfamily PKC [1]. In mammals this superfamily is comprised of 12 isoforms divided in 3 groups depending on their activation requirements: conventional PKC isoforms (α, βI, βII and γ), which activation depends on DAG and Ca^{2+}; novel (δ, ε, η and θ), whose activation depends on DAG but not Ca^{2+} and atypical (λ/ι, μ and ζ), whose activation is independent from both DAG and Ca^{2+} [1]. Once activated by phosphorylation and cofactor binding [2], PKCs stimulate serine/threonine phosphorylation of many cellular proteins including other kinases, cytoskeletal proteins, structural proteins, enzymes, adapter proteins and receptors, and their activation has multiple effects in both normal and pathological processes including differentiation, proliferation, apoptosis, cell death, secretion, adhesion and cell migration [3].

The novel PKCθ isoform, which is the most recently described [4], was originally thought to have a restrictive distribution with high expression in T-cells [4], brain [4] and skeletal muscle [4]. Its activation and effect on various cellular processes have been primarily studied in

Abbreviations: CCK, COOH-terminal octapeptide of cholecystokinin; TPA, 12-O-tetradecanoylphorbol-13-acetate; GI, gastrointestinal; CCK-JMV, CCK-JMV-180; IP, immunoprecipitation; Co-IP, co-immunoprecipitation; PKD, protein kinase D; GPCR, G protein-coupled receptor; PYK2, proline-rich tyrosine kinase 2; FAK, focal adhesion kinase; IKK, IκB kinase; AKT, protein kinase B; VIP, vasoactive intestinal peptide; HGF, hepatocyte growth factor; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor beta; IGF-1, insulin-like growth factor 1; bFGF, basic fibroblast growth factor; PKA, protein kinase A; DAG, diacylglycerol; 8-Br-cAMP, 8-Bromo-cyclic adenosine monophosphate; TCR, T-cell receptor; PLC, phospholipase C; HRP, horseradish peroxidase; MAPK/ERK, mitogen-activated protein kinase; IRS-1, insulin receptor substrate 1; CARMA, caspase recruitment domain (CARD) carrying member of the membrane associated guanylate kinase (MAGUK) family proteins; MALT-1, mucosa-associated lymphoid tissue translocation gene 1; Cbl, Casitas B-lineage lymphoma proto-oncogene; Bcl-10, B-cell lymphoma/leukemia 10 protein; PI3K, phosphatidylinositol-3-; Kinase, CAKβ, cell adhesion kinase β; NFκβ, nuclear factor-kappa B; PAR2, protease-activated receptor 2; PAR4, protease-activated receptor 4

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0167-4889/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.bbamcr.2011.07.007

these tissues, especially in T-cells [5]. Numerous subsequent studies show that PKCθ is more widely distributed than originally described [4] and it has been detected in a number of other tissues such as in testis [4], intestinal epithelial cells [6] and mast cells [7]; and in several human tumor and tumor cell lines [such as human gastrointestinal stromal tumor [8], human colorectal cancer [9] and gastric cancer cells KATO-III [10]].

Studies demonstrate that PKCθ activation plays an important function in various tissues including T cell antigen receptor (TCR) activation, proliferation, apoptosis [5], insulin secretion [11], insulin signaling in muscle [12], barrier permeability in intestinal epithelium [6], thrombus formation in platelets [13] and mast cell activation [7]. However, there is little information on the activation of PKCθ by gastrointestinal (GI) hormones/neurotransmitters and growth factors.

Pancreatic acinar cells are an excellent model system to study kinase activation by GI hormones/neurotransmitters and growth factors because many GI hormones/neurotransmitters and growth factors can alter pancreatic acinar function and signaling cascades including phospholipases (A, C, D), adenylate cyclase, tyrosine kinases and other serine/threonine kinases [14–19]. In pancreatic acinar cells from normals or animals with pancreatic disorders (pancreatitis, pancreatic cancer), PKC activation, including conventional and other novel PKCs (PKCδ and PKCε), has been implicated in several processes. These include enzyme secretion, activation of proteases, inflammatory responses, growth and apoptotic pathways stimulated by various pancreatic hormones/neurotransmitters or growth factors, as well as other stimulants [20–24]. At present, it is unclear whether PKCθ is present in pancreatic acinar cells, whether any pancreatic neurotransmitter/hormones or growth factors can activate it or whether it participates in any of signaling cascades mediating either the physiological or pathological processes caused by pancreatic neurotransmitter/hormones or growth factor stimulation of pancreatic acinar cells.

The purpose of the present study was to address these issues and to determine whether PKCθ is present in pancreatic acinar cells, if gastrointestinal hormones/neurotransmitters can activate this novel protein kinase, PKCθ, and if so, to provide insights into the possible mechanisms of its interactions with various known important cellular mediators of the actions of these pancreatic stimulants. Particular attention was paid to the hormone/neurotransmitter, cholecystokinin (CCK), because it is not only a physiological regulator of pancreatic acinar cell function, it is also important in a number of important pathological processes affecting acinar cell function, such as pancreatitis [23,25,26].

2. Materials and methods

2.1. Materials

Male Sprague–Dawley rats (150–250 g) were obtained from the Small Animals Section, Veterinary Resources Branch, National Institutes of Health (NIH), Bethesda, MD. Rabbit anti-phospho-protein kinase C θ (PKCθ) pT538, rabbit anti-PKCθ, rabbit anti-phospho-Src family (Y416), mouse monoclonal anti-phospho p44/42 mitogen-activated protein kinase (MAPK) (T202/Y204) (E10), rabbit anti-Akt, rabbit anti-RafA, rabbit monoclonal anti-RafB (55C6), rabbit anti-RafC, rabbit anti-protein kinase D (PKD), rabbit anti-protein kinase δ (PKCδ), rabbit anti-14-3-3-γ, rabbit monoclonal anti-Bcl-10 (C78F1), rabbit anti-Mucosa-associated lymphoid tissue translocation gene 1 (MALT-1), rabbit anti-c-Cbl, rabbit anti-phospho Akt (T308), rabbit anti-phospho-IκB kinase (IKK) IKKα (Ser180)/IKKβ (Ser181), rabbit monoclonal anti-phospho Raf C (56A6), rabbit phospho-PKD (Ser744/748), rabbit phospho-FAK (Tyr397), rabbit phospho-Pyk2 (Tyr402), rabbit phospho-PKCδ (Tyr311), rabbit anti-α/β tubulin antibodies and nonfat dry milk were purchased from Cell Signaling Technology, Inc. (Beverly, MA). Mouse monoclonal anti-PKCθ (E-7) antibody, mouse monoclonal anti-Lyn (H-7), mouse anti-pan Src, bovine anti-goat horseradish peroxidase (HRP)-conjugate and anti-rabbit-HRP-conjugate antibodies were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Mouse monoclonal anti-PKCθ (clone 27) was purchased from BD Biosciences (San Jose, CA). Mouse monoclonal anti-cadherin (36/E) antibody was from BD Transduction laboratories (Lexington, KY). Mouse monoclonal anti-calpain (15C10) antibody was from Biosource International, Inc. (Camarillo, CA). Rabbit anti-phosphatidylinositol-3-kinase p85 (PI3K) was purchased from Upstate Biotechnology (Lake Placid, NY). Tris/HCl pH 8.0 and 7.5 were from Mediatech, Inc. (Herndon, VA). 2-mercaptoethanol, protein assay solution, sodium lauryl sulfate (SDS) and Tris/Glycine/SDS (10×) were from Bio-Rad Laboratories (Hercules, CA). $MgCl_2$, $CaCl_2$, Tris/HCl 1 M pH 7.5 and Tris/Glycine buffer (10×) were from Quality Biological, Inc. (Gaithersburg, MD). Minimal essential media (MEM) vitamin solution, Dulbecco's Modified Eagle Medium (DMEM), Waymouth's medium, basal medium Eagle (BME) amino acids 100×, Dulbecco's phosphate buffered saline (DPBS), glutamine (200 mM), Tris–Glycine gels, L-glutamine, fetal bovine serum (FBS), 0.05% trypsine/EDTA solution, penicillin–streptomycin, Alexa 594, Alexa 488-conjugated anti-rabbit secondary antibodies and glycerol were from Invitrogen (Carlsbad, CA). COOH-terminal octapeptide of cholecystokinin (CCK), hepatocyte growth factor (HGF), bombesin, insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), vasoactive intestinal peptide (VIP), endothelin and secretin were from Bachem Bioscience Inc. (King of Prussia, PA). CCK-JMV-180 (CCK-JMV) was obtained from Research Plus Inc., Bayonne, NJ. Epidermal growth factor (EGF), thapsigargin, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), deoxycholic acid, protein kinase C isoenzyme sample kit and myristolated PKCθ pseudosubstrate were from Calbiochem (La Jolla, CA). Carbachol, insulin, transforming growth factor beta (TGFβ), dimethyl sulfoxide (DMSO), 12-O-tetradecanoylphobol-13-acetate (TPA), 8-bromoadenosine 3′5′ cyclic monophosphate sodium (8-Bromo-cAMP), L-glutamic acid, glucose, fumaric acid, pyruvic acid, trypsin inhibitor, HEPES, TWEEN® 20, Triton X-100, phenylmethanesulfonylfluoride (PMSF), ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), sucrose, sodium-orthovanadate, sodium azide, Nonidet P40, sodium pyrophosphate, β-glycerophosphate, sodium fluoride, dithiothreitol, AEBSF, MOPS (3-(N-morpholino)propanesulfonic acid), methanol and CeLytic™M Cell Lysis Reagent were from Sigma-Aldrich, Inc. (St. Louis, MO). Albumin standard, Protein G agarose beads and Super Signal West (Pico, Dura) chemiluminescent substrate were from Pierce (Rockford, IL). Protease inhibitor tablets, pepstatin, leupeptine and aprotine were from Roche (Basel, Switzerland). Purified collagenase (type CLSPA) was from Worthington Biochemicals (Freehold, NJ). Nitrocellulose membranes were from Schleicher and Schuell Bioscience, Inc. (Keene, NH). Biocoat collagen I Cellware 60 mm dishes were from Becton Dickinsen Labware (Bedford, MA). Albumin bovine fraction V was from MP Biomedical (Solon, OH). NaCl, KCl, acetone, phosphoric acid and NaH_2PO_4 were from Mallinckrodt (Paris, KY). HEK 293 cells were from ATCC (Manassas, VA). Dominant negative PKCθ adenovirus, Quick Titer™ Adenovirus Quantification Kit and ViraBind™ Adenovirus Purification Kit were from Cell Biolabs, Inc. (San Diego, CA). Ad-CMV-Null was from Vector Biolabs (Philadelphia, PA). RNA PCR Kit, DNA-polymerase (Amplitaq Gold), 10× PCR buffer and deoxynucleotides were from Applied Biosystems (Foster City, CA). L-364,718 (3S(−)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepine-3-yl-1H-indole-2-carboxamide) and L-365,260 (3R(+)-N-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N′-(3-methylphenyl)urea) were from Merck, Sharp and Dohme (West Point, PA). YM022 ((R)-1-[2,3-dihydro-1-(2′-methyl-phenacyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl) urea) and SR27897 (1-[[2-(4-(2-chlorophenyl)-thiazol-2-yl)amino-carbonyl] indolyl]acetic acid were from Tocris Bioscience (Ellisville, MO). Phadebas reagent was from Magle Life Science (Lund, Sweden). PKC Assay Kit and Histone H1 were from Millipore (Temecula, CA). [γ-^{32}P]ATP (3000 Ci/mmol) was from Perkin Elmer (Waltham, MA).