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Estrogen induces phospholipase A_2 activation through ERK1/2 to mobilize intracellular calcium in MCF-7 cells

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ABSTRACT

The principal secreted estrogen, 17β-estradiol rapidly activates signaling cascades that regulate important physiological processes including ion transport across membranes, cytosolic pH and cell proliferation. These effects have been extensively studied in the MCF-7 estrogen-responsive human breast carcinoma cell line. Here, we demonstrate that a physiological concentration of 17β-estradiol caused a rapid, synchronous and transient increase in intracellular calcium concentration in a confluent monolayer of MCF-7 cells 2–3 min after treatment. This response was abolished when cells were pre-incubated with the phospholipase A_2 (PLA_2) inhibitor quinacrine or with the cyclooxygenase inhibitor indomethacin. The translocation of GFP-c$PLA_2\alpha$ to perinuclear membranes occurred 1–2 min after 17β-estradiol treatment; this translocation was concurrent with the transient phosphorylation of c$PLA_2\alpha$ at serine residue 505. The phosphorylation and translocation of cPLA_2 were sensitive to inhibition of the extracellular signal regulated kinase (ERK) signaling cascade and occurred simultaneously with a transient activation of ERK. The phosphorylation of cPLA_2 could be stimulated by membrane impermeable 17β-estradiol conjugated to bovine serum albumen and was blocked by an antagonist of the classical estrogen receptor. Here we show, for the first time, that PLA_2 and the eicosanoid biosynthetic pathway are involved in the 17β-estradiol induced rapid calcium responses of breast cancer cells.

1. Introduction

The most active, secreted form of estrogen, 17β-estradiol (E_2) modulates whole body physiology through endocrine, autocrine and paracrine mechanisms to elicit diverse responses in different target tissues [1,2]. The physiological importance of E_2 is emphasized when normal circulatory E_2 levels are perturbed, contributing to the development and progression of many diseases. The role of E_2 in normal bone recycling and the consequences of its withdrawal at menopause leading to osteoporosis are established [3]. E_2 also has proliferative effects on breast and primary reproductive tissues [4]. Recent epidemiological evidence links the reinstatement of E_2 through hormone replacement therapy to an increased risk of breast cancer initiation and progression [5]. The contribution of E_2 to breast cancer development has been the subject of intense investigation and much information about the E_2 linked cell signaling responses have been gained by studying its effects on model breast cancer cell lines. At the cellular level, E_2 acts through two interlinked mechanisms: The genomic, transcriptional response mediated by classical E_2 receptors [6,7] and also diverse rapid, cell signaling processes whose receptor linkage is not yet fully elucidated [7–9].

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The E_2 stimulated changes in ion transport across cell membranes that culminate in cytoplasmic alkalinization [10,11] and an increase in intracellular Ca^{2+} levels [12–14] make a major contribution to overall cellular physiology including proliferation. The proliferative effects of E_2 are regulated through multiple pathways; however, the sustained activation of the extracellular signal regulated kinase (ERK1/2) mitogen activated protein kinase (MAPK) signaling pathway is the best studied and has been implicated in the development of E_2 deprivation resistant breast cancer [15]. At least in MCF-7 cells the initiation of ERK1/2 activation 3–5 min after E_2 application appears to be calcium dependent [16–18]. The signaling pathways by which E_2 raises intracellular calcium levels are not fully elucidated; however, we have previously demonstrated a PKA and PKCδ dependency in the rapid calcium influx stimulated by E_2 in the colon [19–21]. Significantly, PKCδ is involved in regulating the ERK1/2-dependent growth promotion of MCF-7 cells following E_2 treatment [16]. E_2 also rapidly increases adenylate cyclase activity in breast cancer cells, isolated uterine cells and also in the intact uterus with a concurrent stimulation of CRE-dependent transcription [22].

The arachidonic acid (AA) based eicosanoid signaling pathway is also involved in the rapid E_2 responses of the colon [23], embryonic membranes [24] and also in rapid effects of 1α,25-$(OH)_2D_3$ but not E_2 on chondrocytes [25]. Both the inhibition of phospholipase A_2 (PLA_2) with quinacrine and the inhibition of the cyclooxygenase (COX) family enzymes with indomethacin block the calcium response to E_2 in the isolated colonic crypts [10]. The different isoforms of PLA_2 release AA from membrane phospholipids while the COX enzymes catalyze the conversion of AA to prostaglandin H_2 (PGH_2) [26]. PGH_2 is a substrate for prostaglandin E_2 synthase (PGES) [27,28] that produces PGE_2 and other biologically active prostaglandins through further metabolism. Prostaglandins regulate the inflammatory response and other physiological processes through the activation of G-protein coupled receptors [29].

PLA_2 acts on the sn-2 bond of membrane phospholipids to release AA and a lysophospholipid. Both of these products are potent secondary messengers [26,30]. The different isoforms of PLA_2 are divided into three groups. The $sPLA_2$ enzymes are secreted from the cell and require extracellular calcium for their activity. The intracellular $iPLA_2$ enzymes are membrane associated or cytosolic and their activation is independent of calcium availability. The calcium dependent $cPLA_2$ enzymes are cytosolic but translocate to intracellular membranes following activation. The $cPLA_2\alpha$ isoform is ubiquitously and constitutively expressed in most cells. Different stimuli that raise intracellular calcium levels promote the activation of $cPLA_2$, including ATP depletion [31] or treatment with noradrenalin [32]. Calcium binding to the C2 domain promotes the translocation of $cPLA_2$ and its association with vimentin, rather than promoting activation [33]. In some experimental systems the translocation of $cPLA_2$ can also occur at resting $[Ca^{2+}]_i$, such as in response to okadaic acid or phorbolmyristylacetate (PMA) [34,35].

Here, we investigated the regulation of the rapid rise in $[Ca^{2+}]_i$ following E_2 treatment in the MCF-7 cell line through $cPLA_2$ activation and AA metabolism based on our previous work in the isolated colonic crypts.

2. Experimental

2.1. Cell culture

The MCF-7 human breast carcinoma cell line (ATTC, Teddington, United Kingdom) was maintained in DMEM:Ham's F12 medium without phenol red (Sigma–Aldrich, Dublin, Ireland) supplemented with non-essential amino acids, sodium pyruvate, L-glutamine and 10% fetal bovine serum (Invitrogen Life Technologies, Paisely, United Kingdom) and propagated at 37 °C in a humid atmosphere of 5% CO_2. Prior to steroid treatment the cells were subjected to progressive serum depletion over 4 days (24 h at 5% serum, 24 h at 2% serum and 48 h serum free) to achieve growth arrest.

2.2. Reagents and antibodies

E_2 (Sigma–Aldrich) was initially dissolved in methanol at a concentration of 10 mM then diluted to a final concentration of 10 nM in serum free tissue culture medium. E_2 conjugated to bovine serum albumen (E_2-BSA) was obtained from Steraloids (Newport, RI) and diluted to a final concentration of 10 nM in serum free tissue culture medium immediately before each experiment. The phospholipase A inhibitor quinacrine was from Sigma–Aldrich as was the cyclooxygenase inhibitor indomethacin. The ER antagonist ICI 182,780 and the MAPK kinase (MEK) inhibitor PD98059 were from Tocris (Avonmouth, United Kingdom). All other chemical reagents used in this investigation were obtained from Sigma–Aldrich, unless otherwise specified.

The primary polyclonal antibodies used in this investigation, anti-phosphoSer505 $cPLA_2$, total $cPLA_2$, anti-phosphoThr202/phosphoTyr204 ERK1/2 and total ERK1/2 were from Cell Signalling Technology (Hitchin, United Kingdom). The anti-rabbit IgG horseradish peroxidase conjugate used in Western detection was from Sigma–Aldrich. The enhanced chemiluminescene (ECL) detection reagents were from Amersham (Little Chalfont, United Kingdom).

2.3. Analysis of $cPLA_2$ phosphorylation

Subconfluent MCF-7 cells were propagated on 6 cm tissue culture dishes and subjected to progressive serum depletion over 96 h prior to treatment with 10 nM E_2 or methanol vehicle control over a period of 5 or 10 min. At the indicated time points, the plates were placed on ice and the cells washed with ice-cold phosphate buffered saline (PBS). The cells were lysed in Laemmli sample buffer and the proteins from the lysates were separated on 8% SDS-PAGE gels then transferred onto PVDF membrane (Amersham). The membranes were probed with anti-phosphoSer505 $cPLA_2$ or anti-$cPLA_2$ polyclonal rabbit antibody. Binding of the primary antibody was detected using an anti-rabbit horseradish peroxidase conjugate and visualized using ECL. The increase in phosphorylation was quantified by densitometry using Genesnap software (Synoptics Ltd., Cambridge, United Kingdom). The mean densitometry data from at least three independent experiments are presented as fold increases relative to the values of untreated controls. The analysis of variance (ANOVA) of