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Sphingosine-1-phosphate receptor 1 transmits estrogens' effects in endothelial cells

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ABSTRACT

We have previously reported that the steroid hormone estrogens stimulate activation of sphingosine kinase 1 (SphK1) and sphingosine-1-phosphate (S1P) receptors in breast cancer cells. Both estrogens and S1P are potent biological modulators of endothelial function in vasculature able to activate multiple effectors, including endothelial nitric oxide synthase (eNOS). In this study we report that treatment of endothelial cells (ECs) with 17β-estradiol (E_2) resulted in a rapid, transient, and dose-dependent increase in SphK activity and increased S1P production. The effect was not reproduced by the inactive E_2 analogue 17α-E_2. Expression of the dominant-negative mutant SphK1^{G82D} or transfection of SphK1-targeted siRNA in ECs caused not only a defect in SphK activation by E_2, but also a significant inhibition of E_2-induced activation of Akt/eNOS. Furthermore, E_2 treatment induced internalization of plasma membrane S1P1 receptor, accompanied with an increase in the amount of cytosolic S1P1. By down-regulating S1P1 receptor expression, the S1P1-specific antisense oligonucleotides significantly inhibited E_2-induced activation of Akt/eNOS in ECs. E_2-induced EC migration and tube formation were also inhibited by S1P1 down-regulation. Thus, the findings indicate an important role of the SphK1/S1P1 pathway in mediating estrogen signaling and its actions in vasculature.

1. Introduction

Multiple estrogen-dependent physiological effects have been demonstrated in vascular endothelium since estrogen receptors (ER) were detected in cardiovascular tissue more than three decades ago [1]. For instance, estrogens are essential for pregnancy-related angiogenesis and intense blood flow in the uterine vascular system and placenta [2,3]. Accordingly, reduction of flow-mediated dilation in women becomes apparent at the age of menopause with a dramatic decrease in circulatory estrogens, pointing to an active vasodilatory role of the hormone [4]. In postmenopausal women, chronic treatment with estrogens facilitates endothelium-dependent vasodilation in large peripheral arteries [5].

It was suggested that this vasodilatory effect is mediated by a rapid non-genomic action of estrogens [6,7]. Notably, estrogens can switch on functioning of the endothelial isoform of nitric oxide (NO) synthase (eNOS) and increase NO production in vasculature through several signaling pathways including activation of phosphatidylinositol-3-OH kinase (PI3K)/Akt, cSrc and MAP kinases [7,8]. Both ERα and ERβ were shown to be coupled to eNOS in caveolae, a subset of lipid rafts known to serve as a compartment for signaling molecules [9,10]. The interaction of extra-nuclear ER with the G-proteins and/or with heat shock protein 90 (HSP90) provided yet another mechanism of estrogen-induced vasodilation and activation of eNOS [11]. In addition, an important role of c-Src has been proposed for the realization of this signaling event involving the smaller variant ERα46 [12].

Sphingolipid signaling has been recently demonstrated as a key part amongst the rapid estrogen-activated pathways [13–19]. We have previously reported that estrogens can stimulate sphingosine kinase 1 (SphK1) and increase intracellular production of

Abbreviations: SphK1, sphingosine kinase 1; S1P1, sphingosine-1-phosphate (S1P) receptor 1; ECs, endothelial cells; E_2, 17β-estradiol; ER, estrogen receptor; Akt, protein kinase B; eNOS, endothelial nitric oxide (NO) synthase; HUVEC, human umbilical vein endothelial cells; BAEC, bovine aortic endothelial cells; HSP90, heat shock protein 90; VEGF, vascular endothelial growth factor; PDGF, platelet-derived growth factor; PTX, pertussis toxin.

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sphingosine-1-phosphate (S1P) [13], the latter in turn activates its own receptors to signal the non-genomic actions of estrogens in human breast cancer cells [17]. Cumulative evidence suggests that some sphingolipids, and specifically S1P, which is abundantly present in the circulation, plays a primary role in regulating blood flow and angiogenesis [19–21]. Interestingly, a recent clinical study showed that the plasma S1P levels were significantly higher in women than in men within the age of 16–55 years old; and higher in pre-menopausal than post-menopausal women, suggesting a correlation between circulating estrogens and S1P levels [22].

In isolated mouse aortas, S1P triggered vessel relaxation via activation of the S1P3 receptor, which was abolished in eNOS deficient mice [21]. This finding supports the notion that S1P has an anti-atherosclerotic effect, which is attributable to NO production and estrogens' action [23]. The downstream signaling pathways of S1P receptors include a wide range of kinases, including MEK, ERK1/2, p38 [24], c-jun N-terminal kinase [25], PI3 kinase, and focal adhesion kinase [26,27]; all of them were also linked to eNOS signaling [28].

The involvement of S1P-initated pathways up- and downstream of NO production has been demonstrated previously by several studies [19,29,30]. S1P can initiate not only activation of eNOS, but also reinforce its activation triggered by other factors. Cytoplasmic cascades activated by S1P receptors in endothelial cells have been shown to engage in crosstalk with multiple signaling pathways of cytokines and growth factor receptors. Tanimoto and co-workers identified a trans-activation of vascular endothelial growth factor (VEGF) receptor by S1P [31]. These authors showed that inhibition of the tyrosine kinase activity of the VEGF receptor reduces S1P-stimulated eNOS phosphorylation. Other groups [32–34] found an interaction of S1P signaling with PDGF receptor in different models. Kwon and co-workers reported that the protective eNOS-linked effects of S1P were dependent on activation of the receptor S1P1 and S1P3 [35]. It was also demonstrated that TNFα induced activation of eNOS was mediated via S1P receptors [36]. Collectively, it appears that a strong connection exists between eNOS and S1P receptor activation in endothelial cells. It is thus intriguing to consider the potential interactions between the signaling of S1P and estrogens and activation of eNOS in the endothelium. Accordingly, preliminary work indicated on important role of S1P receptor signaling in regulation of dilation in mesenteric resistance arteries from aged female rats [37]. Although, whether estrogens are stimulators of the SphK/S1P signaling in ECs has never been tested, nor has the role of sphingolipids as mediators of estrogen-dependent induction of eNOS. In the present study we have addressed the hypotheses that estrogens can activate S1P signaling in endothelial cells and that S1P receptor(s) can mediate estrogen-induced activation of eNOS in these cells.

2. Materials and methods

2.1. Chemicals

S1P and D-*erythro*-sphingosine were purchased from Biomol Research Laboratories Inc. (Plymouth Meeting, PA). S1P was dissolved in methanol (0.5 mg/ml) and then reconstituted in 1% fatty acid free BSA with sonication to make a stock solution at 2 mmol/liter stored at −70 °C until use. PTX, ATP, E_2, and 17α-estradiol were from Sigma–Aldrich (Melbourne, Australia). ICI182,780 was

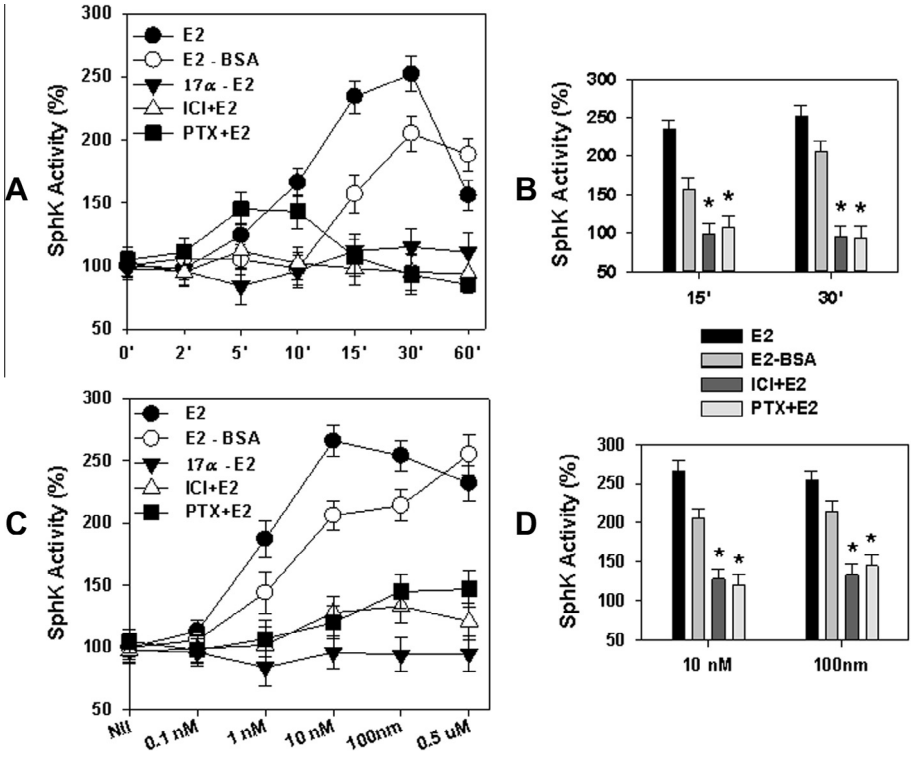

Fig. 1. Estrogens activate SphK in BAEC. (A) Time-dependent effect of E_2 on SphK activity. SphK activity was measured in BAEC treated with 100 nM E_2 alone or in the presence of inhibitors (PTX or ICI), or 100 nM 17α-E_2, or 100 ng/ml E_2-BSA for the indicated time course. BAEC were pretreated with 50 ng/ml PTX for 16 h or with 10 μM ICI for 1 h prior application of E_2. (B) Data from three independent experiments with different agents shown in (A) after 15 or 30 min of treatment were pooled and tested for statistical significance using *t*-test. (C) Dose-dependent effects of E_2 on SphK activity. BAEC were treated with increasing doses of agents as described in (A) for 15 min. (D) Data from three independent experiments of (C) and applied in concentrations of 10 or 100 nM were pooled and tested for statistical significance using *t*-test. Data are the means ± SEM of triplicate determinations and are representative of at least three independent experiments; $^{*}p < 0.05$ for the presence vs absence of PTX or ICI analyzed with ANOVA.