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Estrogen-mediated protection of the vascular endothelium requires Notch1

ABSTRACT: Premenopausal women benefit from cardiovascular protection compared to age-matched men. Estrogens exert a protective action against endothelial cells (ECs) apoptosis, one of the hallmarks of endothelial dysfunction leading to cardiovascular disorders, but the molecular mechanisms underlying this effect remain poorly understood. The inflammatory cytokine tumor necrosis factor α (TNFα) causes ECs apoptosis while dysregulating the Notch pathway, a major regulator of ECs survival. We have previously reported that treatment with 17β-estradiol (E2) activates Notch signaling in ECs. Based on the observation that TNFα and E2 have opposite effects both on ECs apoptosis and on Notch signaling, the aim of this study was to determine whether, under inflammatory conditions, Notch is involved in E2-mediated protection against endothelial cells apoptosis. With this aim, we evaluated also the possible role of estrogen receptor (ER) α and/or β in the E2-mediated action. Human umbilical vein endothelial cells (HUVECs) were treated with E2 and/or TNFα and the effects on apoptosis and on the Notch pathway were investigated. We found that TNFα-induced apoptosis was counteracted by E2. When Notch1 was inhibited, the E2-mediated protection was not observed, whereas Notch1 ectopic overexpression diminished TNFα-induced apoptosis. In addition, TNFα reduced the levels of active Notch1 protein, which were partially restored by E2 treatment. Furthermore, we show that TNFα-mediated Akt phosphorylation is Notch1-dependent and E2 enhances this effect. Moreover, treatment with PHTPP (ERβ-antagonist) or with siRNA against ERβ or with PPT (ERα-agonist) abolished E2's effects on apoptosis and on active Notch1. Conversely, treatment with siRNA against ERα or with DPN (ERβ-agonist) did not inhibit the E2-mediated effects on apoptosis and on active Notch1. In summary, the data reported indicate that E2, through a mechanism involving ERβ, requires active Notch1 to protect the vascular endothelium. These findings could be relevant when assessing efficacy and applicability of menopausal hormone treatment, since they indicate that in subjects with impaired Notch signaling, due to pathological conditions, hormone therapy might not provide an effective protection to the vascular endothelium.