ESTROGENIC REGULATION OF ENDOTHELIAL GLYCOLYTIC PATHWAY

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Background and aim: 17β-estradiol (E2) affects multiple aspects of cell metabolism. We recently demonstrated that E2triggers angiogenesis via rapid signaling that requires the membrane estrogen receptor GPER1and up-regulation of the key glycolytic protein PFKFB3as a downstream effector. The activity of PFKFB3and other proteins involved in the glycolytic process, including the glucose transporter 1(GLUT1), is regulated at both transcriptional and post-translational levels, depending on cell type. We investigated the mechanisms of estrogenic regulation of the glycolytic pathway in human endothelial cells (HUVECs).

Methods: HUVECs were isolated from normal-term umbilical cords. PFKFB3and GLUT1mRNA levels were assessed by rtPCR. The involvement of the ubiquitin-proteasome system in PFKFB3degradation as well as the effect of E2and the selective GPER agonist G1on protein stability were evaluated by Western blot, using proteasome and protein synthesis inhibitors respectively; E2-regulated PFKFB3ubiquitination was assessed by indirect immunoprecipitation assay. This approach was also used to assess the effect of estrogens on other glycolytic proteins, including GLUT1and hexokinase 2(HK2).

Results: Treatment with both E2as well as G1concentration-dependently increased PFKFB3protein amounts peaking at 3hours, without affecting mRNA levels. The selective E3ubiquitin ligase (SMER-3) and the proteasome inhibitor MG132increased PFKFB3protein levels after 3-6h. In the presence of the protein synthesis inhibitor cycloheximide, treatment with E2(100 nM) counteracted cycloheximide-mediated PFKFB3degradation over time. Similar findings were obtained with G1(100 nM). We also found that E2and G1enhanced GLUT1expression in a time-dependent manner (1-24h), again without increasing mRNA levels. No differences in HK2protein levels were found.

Conclusion: This is the first evidence showing that E2affects glycolysis by targeting the glycolytic protein PFKFB3stability. These data point to a novel mechanism that links hormonal signals to metabolic demand through the membrane receptor GPER1and suggest a strategy by which female steroid hormones might exert rapid control on metabolic pathways in response to environmental cues.