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THE BASIC FIBROBLAST GROWTH FACTOR STIMULATES NITRIC OXIDE PRODUCTION IN CHO-K1 CELLS: ROLE OF CERAMIDE

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Nitric Oxide (NO) is an important intracellular and intercellular mediator involved in the modulation of different physiological processes, including the regulation of neoangiogenesis. In our work we analyzed the effects of basic fibroblast growth factor (bFGF) on NO production in CHO-K1 cells and the intracellular mechanisms involved. We found that basic FGF induces NO production through the activation of the endothelial form of NO synthase (NOS), causing a subsequent increase in the cGMP levels. It was reported that the activation of eNOS almost always requires the presence of the calmodulin complex. In our cell model, otherwise, bFGF activates the enzyme in a Ca^{++} and MAP kinase-independent manner. The translocation of the eNOS from the plasma membrane, where is located in caveolae bound to caveolin 1, to the cytosol is the crucial step for the synthesis of NO. We demonstrated that this dissociation and the cytosolic translocation of the enzyme is due to the production of ceramide by bFGF's activation of the sphingomyelinase enzyme. In fact in the presence of the acidic sphingomyelinase inhibitor D609 no nitric oxide production is observed. Moreover, immunofluorescence experiments, showed that D609 prevented bFGF dependent eNOS translocation to the cytosol. To support this evidence we evaluated the ceramide concentration using HPLC-mass-spectrometry in control cells and in cells treated with bFGF obtaining, after stimulation, a substantial increase of ceramide synthesis. This sphingolipid is now recognized as an important intracellular second messenger in various cells types and in our cell system it is responsible of a novel Ca^{++}/calmodulin-independent mechanism for eNOS activation. Moreover, the production of this sphingolipid by bFGF leads to CHO-K1 proliferation through NO synthesis. C8:0 ceramide caused a dose-dependent DNA synthesis, measured with the [^{3}H]-thymidine incorporation, up to 100% of proliferation after 2 h of treatment. Investigating the mechanism of proliferation we found out that it follows a MAPK- independent pathway while usually this growth factor mainly exerts its proliferative effect through the MAP-kinase cascade. The treatment with ceramide stimulated cell proliferation that was inhibited by ceramide/ NO/cGMP/PKG blockers. From these results we propose that the two proliferative pathways activated by bFGF converge only at the final physiological effect. In conclusion we describe a completely novel transduction mechanism activated by bFGF receptor leading to cell proliferation through the synthesis of ceramide and nitric oxide.
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