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Expression of phase II detoxifying genes is regulated by NF-E2-related factor 2 (Nrf2)-mediated antioxidant response element (ARE) activation. Phosphatidylinositol 3-kinase (PI3-kinase) plays an essential role in ARE-mediated rGSTA2 induction by oxidative stress and controls microfilaments and translocation of actin-associated proteins. This study was designed to investigate the PI3-kinase-mediated nuclear translocation of Nrf2 and the interaction of Nrf2 with actin. Pretreatment of the cells with PI3-kinase inhibitors (wortmannin/LY294002) prevented nuclear translocation of Nrf2 by tert-Butylhydroquinone (*t*-BHQ). *t*-BHQ relocalized Nrf2 in concert with changes in actin microfilament architecture, as visualized by confocal microscopy. Furthermore, *t*-BHQ increased the level of nuclear actin, co-immunoprecipitated with Nrf2, which returned to that of control by pretreatment with PI3-kinase inhibitors. Cytochalasin B, an actin disruptor, alone stimulated actin-mediated nuclear translocation of Nrf2 and induced rGSTA2. These results were blocked by phalloidin that stabilizes actin filaments. Subcellular fractionation and immunoblot analyses allowed us to detect both 57 kDa and 100 kDa Nrf2. Immunoprecipitation assays showed that the 100 kDa protein comprised both Nrf2 and actin. This study demonstrates that the PI3-kinase regulates rearrangement of actin in response to oxidative stress and that depolymerization of actin causes a complex of Nrf2 to translocate into nucleus.

[PA1-4] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Hydrogen Peroxide Activates ERK in Cultured Feline Ileal Smooth Muscle Cells

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H_2O_2 has been shown to act as a signaling molecule involved in many cellular functions such as oxidant-induced stress, apoptosis, proliferation. In this study, we investigated the action mechanisms of H_2O_2 on activation of Extracellular Signal-Regulated Protein Kinase(ERK) in cultured feline ileal smooth muscle cells(ISMC). Western blot analysis done with phospho-specific MAP kinases antibodies demonstrated that potent activation of ERK and moderate activation of SAPK/JNK occurred within 30 min of H_2O_2 treatment. However, p38 MAP kinase was not activated by H_2O_2. The activation of ERK by H_2O_2 was reduced by MEK inhibitor PD98059, removal of extracellular Ca^{2+}, depletion of the intracellular Ca^{2+} pool by thapsigargin, or pretreatment of ISMC with the calmodulin antagonist W-7. In addition, H_2O_2-induced ERK activation was attenuated by a tyrosine kinase inhibitor genistein, but not by downregulation of protein kinase C(PKC) with phorbol-12-myristate-13-acetate(PMA) or by a PKC inhibitor GF109203X. Further, ERK activation by H_2O_2 was blocked by pretreatment with either *N*-acetyl-cysteine, *o*-phenanthroline, or mannitol. Taken together, these data show the factors controlling MAPK activation by H_2O_2 in intestinal smooth muscle cells and suggest that ERK plays a critical role in the oxidant cell injury induced by H_2O_2.

[PA1-5] [10/18/2002 (Fri) 09:30 - 12:30 / Hall C]

Arachidonic Acid Liberated through Activation of $iPLA_2$ Mediates the Production of Reactive Oxygen Species and Apoptosis Induced by N-Ethylmaleimide in HepG2 Human Hepatoma Cellls

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