Induction of reactive oxygen species by activation of the EP2 receptor contributes to prostaglandin E2-induced cytotoxicity in motor neuron-like NSC-34 cells

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We have shown previously that prostaglandin E2 (PGE2) directly induces neuronal death through activation of the E-prostanoid (EP) 2 receptor in differentiated NSC-34 cells, motor neuron-like cell line. In the present study, to clarify the mechanisms underlying PGE2-induced neurotoxicity, we focused on generation of intracellular reactive oxygen species (ROS) in NSC-34 cells. Dichlorofluorescein fluorescence analysis of PGE2-treated cells showed that intracellular ROS levels increased markedly with time, and that this effect was antagonized by an EP2 antagonist, PF -04418948, but not EP3 antagonist, L-798,106. Although an EP2 agonist, butaprost, mimicked the effect of PGE2, an EP1/EP3 agonist, sulprostone, transiently but significantly decreased the intracellular ROS. MTT reduction assay and lactate dehydrogenase release assay revealed that PGE2- and butaprost-induced cell death were each suppressed by pretreatment with a cell permeable antioxidant, N-acetylcysteine (NAC). Western blot analysis revealed that the active form of caspase-3 was markedly increased in the PGE2- and butaprost-treated cells. These increases in caspase -3 protein expression were suppressed by pretreatment with NAC. Our data have demonstrated that PGE2 is an endogenous inducer of intracellular ROS, and that production of ROS induced by PGE2-EP2 receptor signaling is coupled to the caspase-3 cascade in NSC-34 cells.