

Bradykinin B2 receptor mediates nuclear factor- κ B activation and cyclooxygenase-2 expression via Ras/Raf-1/ERK pathway in human airway epithelial cells

林建煌

Chen BC; Yu CC; Lei HC; Chang MS; Hsu MJ; Huang CL; Chen MC; Sheu JR; Lin CH

Abstract

In this study, we investigated the signaling pathways involved in bradykinin (BK)-induced NF- κ B activation and cyclooxygenase-2 (COX-2) expression in human airway epithelial cells (A549). BK caused concentration- and time-dependent increase in COX-2 expression, which was attenuated by a selective B2 BK receptor antagonist (HOE140), a Ras inhibitor (manumycin A), a Raf-1 inhibitor (GW 5074), a MEK inhibitor (PD 098059), an NF- κ B inhibitor (pyrrolidine dithiocarbamate), and an I κ B protease inhibitor (L-1-tosylamido-2-phenylethyl chloromethyl ketone). The B1 BK receptor antagonist (Lys-(Leu⁸)des-Arg⁹-BK) had no effect on COX-2 induction by BK. BK-induced increase in COX-2-luciferase activity was inhibited by cells transfected with the κ B site deletion of COX-2 construct. BK-induced Ras activation was inhibited by manumycin A. Raf-1 phosphorylation at Ser338 by BK was inhibited by manumycin A and GW 5074. BK-induced ERK activation was inhibited by HOE140, manumycin A, GW 5074, and PD 098059. Stimulation of cells with BK activated I κ B kinase α (IKK α), I κ B α phosphorylation, I κ B α degradation, p65 and p50 translocation from the cytosol to the nucleus, the formation of an NF- κ B-specific DNA-protein complex, and κ B-luciferase activity. BK-mediated increase in IKK α activity and formation of the NF- κ B-specific DNA-protein complex were inhibited by HOE140, a Ras dominant-negative mutant (RasN17), manumycin A, GW 5074, and PD 098059. Our results demonstrated for the first time that BK, acting through B2 BK receptor, induces activation of the Ras/Raf-1/ERK pathway, which in turn initiates IKK α and NF- κ B activation, and ultimately induces COX-2 expression in human airway epithelial cell line (A549).