Lipopolysaccharide enhances bradykinin-induced signal transduction via activation of Ras/Raf/MEK/MAPK in canine tracheal smooth muscle cells

林建煌

Luo SF;Wang CC;Chiu CT;Chien CS;Hsiao LD;Lin CH and Yang CM.

Abstract

Bacterial lipopolysaccharide (LPS) was found to induce inflammatory responses and to enhance bronchial hyperreactivity to several contractile agonists. However, the implication of LPS in the pathogenesis of bronchial hyperreactivity was not completely understood. Therefore, in this study, we investigated the effect of LPS on mitogen-activated protein kinase (MAPK) activation associated with potentiation of bradykinin (BK)-induced inositol phosphates (IPs) accumulation and Ca2+ mobilization in canine cultured tracheal smooth muscle cells (TSMCs).

LPS stimulated phosphorylation of p42/p44 MAPK in a time- and concentration-dependent manner using a Western blot analysis against a specific phosphorylated form of MAPK antibody. Maximal stimulation of the p42 and p44 MAPK isoforms occurred after 7 min-incubation and the maximal effect was achieved with 100 μg ml−1 LPS.

Pretreatment of TSMCs with LPS potentiated BK-induced IPs accumulation and Ca2+ mobilization. However, there was no effect on the IPs response induced by endothelin-1, 5-hydroxytryptamine, and carbachol. In addition, pretreatment with PDGF-BB enhanced BK-induced IPs response.

These enhancements by LPS and PDGF-BB might be due to an increase in BK B2 receptor density (Bmax) in TSMCs, characterized by competitive inhibition of [3H]-BK binding using B1 and B2 receptor-selective reagents.

The enhancing effects of LPS and PDGF-BB were attenuated by PD98059, an inhibitor of MAPK kinase (MEK), suggesting that the effect of LPS may share a common signalling pathway with PDGF-BB in TSMCs.

Furthermore, overexpression of dominant negative mutants, H-Ras-15A and Raf-N4, significantly suppressed p42/p44 MAPK activation induced by LPS and PDGF-BB, indicating that Ras and Raf may be required for activation of these kinases.

These results suggest that the augmentation of BK-induced responses produced by LPS might be, at least in part, mediated through activation of Ras/Raf/MEK/MAPK pathway in TSMCs.