Molecular mechanisms of propofol-involved suppression of nitric oxide biosynthesis and inducible nitric oxide synthase gene expression in lipopolysaccharide-stimulated macrophage-like Raw 264.7 cells

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Abstract

Propofol, a widely used intravenous anesthetic agent, has been reported to have immunosuppressive and antioxidative effects. Nitric oxide (NO) plays crucial roles in mediating inflammatory reactions. This study was designed to evaluate the effects of propofol on regulation of NO synthesis and its possible signal-transducing mechanisms in lipopolysaccharide (LPS)-activated macrophage-like Raw 264.7 cells. Exposure of Raw 264.7 cells to LPS significantly increased nitrite production, but propofol reduced such enhancement in concentration- and time-dependent manners. In parallel, treatment of Raw 264.7 cells with a clinically relevant concentration of propofol (50 muM) significantly inhibited inducible NO synthase (iNOS) mRNA and protein production in LPS-activated Raw 264.7 cells. Propofol at 50 muM decreased the LPS-caused augmentation in nuclear c-Jun levels. An electrophoretic mobility shift assay revealed that propofol significantly decreased the binding affinity of the nuclear extracts from LPS-treated Raw 264.7 cells to activator protein-1 (AP-1) consensus DNA elements. A reporter gene assay further showed that propofol ameliorated the transactivated activity of AP-1 in LPS-stimulated Raw 264.7 cells. LPS sequentially increased phosphorylation of MEK4 and JNK1/2. Meanwhile, a therapeutic concentration of propofol significantly decreased the activation of these two protein kinases. Application of toll-like receptor 4 (TLR4) small interfering (si)RNA to Raw 264.7 cells decreased cellular TLR4 levels and LPS-caused activation of MEK4. Co-treatment with propofol and TLR4 siRNA synergistically lowered the LPS-induced increased biosynthesis of iNOS mRNA and nitrite. Therefore, a clinically relevant concentration of propofol can inhibit NO production and iNOS gene expression in LPS-activated Raw 264.7 cells. The suppressive mechanisms may occur through sequential downregulation of TLR4/MEK4/JNK1/2/AP-1 activation.