Pyrimidinoceptor-mediated potentiation of inducible nitric-oxide synthase induction in J774 macrophages: role of intracellular calcium

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摘要

Abstract

We have shown that, in murine J774 macrophages, binding of UTP to pyrimidinoceptors stimulates phosphoinositide (PI) breakdown and an increase in [Ca2+]i. In this study, UTP modulation of the expression of inducible nitric-oxide synthase (iNOS) was investigated. Although UTP alone had no effect, stimulation of J774 cells with a combination of UTP (10-300 microM) and LPS (0.1-3 microgram/ml) resulted in a potentiated increase in nitrite levels. In parallel, the amount of iNOS protein induced by LPS was also potentiated by UTP treatment. The UTP potentiating effect was attenuated by U73122, suggesting involvement of the downstream signaling pathways of phosphatidylinositide turnover. The tyrosine kinase inhibitor genistein inhibited both the LPS-induced nitrite response and the UTP potentiation. Conversely, two protein kinase C inhibitors, Ro 31-8220 and Go 6976, and a phosphatidylcholine-specific phospholipase C inhibitor, D609, inhibited LPS-stimulated nitrite induction, but did not affect the potentiating effect of UTP, which was also unaffected by pretreatment with phorbol 12-myristate 13-acetate for 8 h. Furthermore, the UTP-induced potentiation was abolished by BAPTA/AM or KN-93 (a selective inhibitor of Ca2+/calmodulin-dependent protein kinase (CaMK)). Nitrite potentiation and iNOS induction were prominent when UTP was added simultaneously with LPS, with the potentiating effect being lost when UTP was added 3 h after treatment with LPS. Pyrrolidinedithiocarbamate (3-30 microM), an inhibitor of NF-kappaB, caused a concentration-dependent reduction in the nitrite response to LPS and UTP. In electrophoretic mobility shift assays, LPS produced marked activation of NF-kappaB and AP-1, which was potentiated by UTP. LPS-induced degradation of IkappaB-alpha as well as the phosphorylation of IkappaB-alpha were

also increased by UTP. Moreover, the UTP-potentiated activation of NF-kappaB and AP-1 and the degradation and phosphorylation of IkappaB-alpha were inhibited by KN-93. Taken together, these data demonstrate that nucleotides, especially UTP, can potentiate the LPS-induced activation of NF-kappaB and AP-1 and of iNOS induction via a CaMK -dependent pathway and suggest that the UTP-dependent up-regulation of iNOS may constitute a novel element in the inflammatory process

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