Involvement of protein kinase C in the UTP-mediated potentiation of cyclic AMP accumulation in mouse J774 macrophages

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摘要

Abstract

1. We have investigated the effects of nucleotide analogues on cyclic AMP formation in mouse J774 macrophages and the mechanisms involved. 2. UTP, in the concentration range 0.1-100 microM, induced concentration-dependent potentiation of prostaglandin E1 (PGE1)-induced cyclic AMP formation, but had no effect on basal cyclic AMP formation. UDP showed an equal potency, while 2-methylthio ATP, alpha, beta-methylene ATP and beta,gamma-methylene ATP gave either a slight increase or had no effect at concentrations up to 100 microM. ATP, although 100 fold less effective than UTP, also caused cyclic AMP potentiation, but had no effect on agonist-stimulated or basal cyclic AMP levels. 3. The cyclic AMP potentiation effect of UTP correlated with increased [Ca2+]i and inositol phosphate (IP) formation over the same concentration range. 4. Ionomycin, which evokes an increase in [Ca2+]i without affecting IP formation, did not cause an increase in cyclic AMP content, indicating that UTP-induced cyclic AMP regulation is not due to activation of Ca(2+)-sensitive adenylyl cyclase isoforms. 5. Although reduced, UTP potentiation was seen in cells incubated in a Ca(2+)-free and/or BAPTA-containing medium. Under these conditions, the UTP-increased IP accumulation was similarly reduced. 6. Exposure of cells to phorbol 12-myristate 13-acetate (PMA) also increased PGE1 stimulation of cyclic AMP levels, and the UTP-induced potentiation of cyclic AMP formation was inhibited by either staurosporine or Ro 31-8220. Pretreatment of cells with PMA for 4-24 h resulted in marked attenuation of UTP-stimulated cyclic AMP potentiation. 7. Pretreatment with pertussis toxin (24 h, 100 ng ml-1) did not significantly affect UTP-induced cyclic AMP potentiation and IP formation, although it increased the cyclic AMP response to PGE1. 8. Analysis of J774 cells by Western

blotting with antibodies specific for different protein kinase C (PKC) isoforms shows the presence of the beta I, beta II, delta, epsilon, eta, mu, lambda and zeta isoforms. Moreover, UTP significantly increased the level of PKC beta I, beta II, delta, epsilon, mu, lambda and zeta immunoreactivity in the membrane fraction and decreased the cytosolic reactivity of PKC beta II, delta, epsilon and zeta. 9. Immunoblot studies also indicate the presence of type II adenylyl cyclase. 10. These results indicate that PKC is required for the potentiation of adenylyl cyclase activity by macrophage pyrimidinoceptors, which exhibit a higher specificity for UTP and UDP than for ATP.