Pharmacological comparison of UTP- and thapsigargin-induced arachidonic acid release in mouse RAW 264.7 macrophages

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摘要

Abstract

1.Although stimulation of mouse RAW 264.7 macrophages by UTP elicits a rapid increase in intracellular free Ca2+ ([Ca2+]i), phosphoinositide (PI) turnover, and arachidonic acid (AA) release, the causal relationship between these signalling pathways is still unclear. In the present study, we investigated the involvement of phosphoinositide-dependent phospholipase C (PI-PLC) activation, Ca2+ increase and protein kinase activation in UTP-induced AA release. The effects of stimulating RAW 264.7 cells with thapsigargin, which cannot activate the inositol phosphate (IP) cascade, but results in the release of sequestered Ca2+ and an influx of extracellular Ca2+, was compared with the effects of UTP stimulation to elucidate the multiple regulatory pathways for cPLA2 activation.

2.In RAW 264.7 cells UTP (100μM) and thapsigargin (1μM) caused 2 and 1.2 fold increases, respectively, in [3H]-AA release. The release of [3H]-AA following treatment with UTP and thapsigargin were non-additive, totally abolished in the Ca2+-free buffer, BAPTA (30μM)-containing buffer or in the presence of the cPLA2 inhibitor MAFP (50μM), and inhibited by pretreatment of cells with pertussis toxin (100ngml−1) or 4-bromophenacyl bromide (100μM). By contrast, aristolochic acid (an inhibitor of sPLA2) had no effect on UTP and thapsigargin responses.

3.U73122 (10μM) and neomycin (3mM), inhibitors of PI-PLC, inhibited UTP-induced IP formation (88% and 83% inhibition, respectively) and AA release (76% and 58%, respectively), accompanied by a decrease in the [Ca2+]i rise.

4.Wortmannin attenuated the IP response of UTP in a concentration-dependent manner (over the range 10nM–3μM), and reduced the UTP-induced AA release in parallel. RHC 80267 (30μM), a specific diacylglycerol lipase inhibitor, had no effect

on UTP-induced AA release.
5.Short-term treatment with PMA (1μM) inhibited the UTP-stimulated accumulation of IP and increase in [Ca2+]i, but had no effect on the release of AA. In contrast, the AA release caused by thapsigargin was increased by PMA.
6.The role of PKC in UTP- and thapsigargin-mediated AA release was shown by the blockade of these effects by staurosporine (1μM), Ro 31-8220 (10μM), Go 6976 (1μM) and the down-regulation of PKC.
7.Following treatment of cells with SK&F 96365 (30μM), thapsigargin-, but not UTP-, induced Ca2+ influx, and the accompanying AA release, were down-regulated.
8.Neither PD 98059 (100μM), MEK a inhibitor, nor genistein (100μM), a tyrosine kinase inhibitor, had any effect on the AA responses induced by UTP and thapsigargin.
9.We conclude that UTP-induced cPLA2 activity depends on the activation of PI-PLC and the sustained elevation of intracellular Ca2+, which is essential for the activation of cPLA2 by UTP and thapsigargin. The [Ca2+]i-dependent AA release that follows treatment with both stimuli was potentiated by the activity of protein kinase C (PKC). A pertussis toxin-sensitive pathway downstream of the increase in [Ca2+]i was also shown to be involved in AA release.