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PLC Mediates Spontaneous Glutamate Release Triggered by Extracellular Calcium

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Keywords

Calcium-sensing receptor, PLC, spontaneous release, mEPSC, calcium

Abstract

Chemical neurotransmission is the major form of communication between neurons and is essential for CNS function. Glutamate is an excitatory fast-acting neurotransmitter that mediates inter-neuronal communication. Evoked and spontaneous release of neurotransmitter are proposed to have distinct roles and rely on divergent vesicle pools and receptors. Moreover, changes in extracellular [Ca2+] ([Ca2+]o) are shown to differentially affect the probability of release for each mechanism. Ca2+ entry via voltage-gated calcium channels (VGCCs) is responsible for evoked release however the same pathway does not contribute to spontaneous release. How does extracellular [Ca2+]o stimulate spontaneous release? Previous experiments demonstrate that the G-protein coupled receptor (GPCR), the calcium-sensing receptor (CaSR), is involved and accounts for ~30% of basal miniature excitatory postsynaptic currents (mEPSCs). Downstream of GPCRs, phospholipase C (PLC) is activated by G protein subunits and hydrolyzes the phospholipid phosphatidylinositol 4,5-biphosphate (PIP2), resulting in inositol triphosphate (IP3) and diacylglycerol (DAG), both of which affect mEPSC frequency. Using whole cell patch clamp in primary neocortical neuronal cultures, we determined that median mEPSC frequency was increased (270% of baseline, n=13) by elevation of [Ca2+]o from physiological (1.1 mM) to high (6.0 mM). Inhibition of PLC with U73122 (5 µM) substantially reduced the sensitivity of mEPSC frequency (95% of baseline, n=8) to the [Ca2+]o increment compared to controls (ANOVA P=0.0002, Dunnett's multiple comparisons, P<0.001), though vehicle (n=14, P>0.99) and the inactive analog, U73343 (n=13, P=0.3295), did not. In PLC1B null mutant mice (n=10), the effect of high [Ca2+]o on mEPSC frequency was attenuated 31% (Dunnett's, P=0.045), and the residual response to high calcium was still reduced by U73122 (n=9, Dunnett's, P<0.01). Taken together these data indicate that PLC strongly links changes in [Ca2+]o to mEPSC frequency and that a substantial fraction of this is mediated by isoform PLC1B.

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