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Original Paper

Disassembly of Subplasmalemmal Actin Filaments Induces Cytosolic Ca^{2+} Increases in *Astropecten aranciacus* Eggs

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Key Words
Actin • Calcium • Fertilization • Phospholipase C • Inositol trisphosphate • Latrunculin

Abstract
Background/Aims: Eggs of all animal species display intense cytoplasmic Ca^{2+} increases at fertilization. Previously, we reported that unfertilized eggs of *Astropecten aranciacus* exposed to an actin drug latrunculin A (LAT-A) exhibit similar Ca^{2+} waves and cortical flashes after 5-10 min time lag. Here, we have explored the molecular mechanisms underlying this unique phenomenon. ***Methods:*** Starfish eggs were pretreated with various agents such as other actin drugs or inhibitors of phospholipase C (PLC), and the changes of the intracellular Ca^{2+} levels were monitored by use of Calcium Green in the presence or absence of LAT-A. The concomitant changes of the actin cytoskeleton were visualized with fluorescent F-actin probes in confocal microscopy. ***Results:*** We have shown that the LAT-A-induced Ca^{2+} increases are related to the disassembly of actin filaments: *i)* not only LAT-A but also other agents depolymerizing F-actin (i.e. cytochalasin B and mycalolide B) induced similar Ca^{2+} increases, albeit with slightly lower efficiency; *ii)* drugs stabilizing F-actin (i.e. phalloidin and jasplakinolide) either blocked or significantly delayed the LAT-A-induced Ca^{2+} increases. Further studies utilizing pharmacological inhibitors of PLC (U-73122 and neomycin), dominant negative mutant of PLC-ɣ, specific sequestration of PIP2 (RFP-PH), $InsP_3$ uncaging, and quantitation of endogenous $InsP_3$ all indicated that LAT-A induces Ca^{2+} increases by stimulating PLC rather than sensitizing $InsP_3$ receptors. In support of the idea, it bears emphasis that LAT-A timely increased intracellular contents of $InsP_3$ with concomitant decrease of PIP2 levels in the plasma membrane. ***Conclusion:*** Taken together, our results suggest that suboolemmal actin filaments may serve as a scaffold for cell signaling and modulate the activity of the key enzyme involved in intracellular Ca^{2+} signaling.

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