SUMMARY OF Ph.D. DISSERTATION

School Fundamental Science and Technology

Student Identification Number

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Title

The mechanism of acrosome reaction cooperatively induced by ARIS and asterosap in starfish sperm

Abstract

In the starfish, *Asterias amurensis*, the cooperation of three components of the egg jelly, i.e. ARIS (acrosome reaction-inducing substance), Co-ARIS and asterosap, is responsible to induce the acrosome reaction. Experimentally, ARIS and asterosap are sufficient for the reaction. Asterosap transiently increases the intracellular concentration of cGMP ([cGMP]_i), through the activation of the asterosap receptor, a guanylyl cyclase, and then increases the intracellular pH (pH_i) and intracellular concentration of Ca^{2+} ([Ca^{2+}]_i).

In this study, we showed that the sperm became unresponsive to the egg jelly to undergo the acrosome reaction when they are pretreated with asterosap. However, in the presence of IBMX or zaprinast, inhibitors against phosphodiesterases (PDEs), sperm retained the capacity to undergo the acrosome reaction in response to the egg jelly even if they were pretreated with asterosap. IBMX and zaprinast kept the [cGMP]_i high after the pretreatment of sperm with asterosap. These results suggest that asterosap-induced elevation of [cGMP]_i is essential for the egg jelly-induced acrosome reaction.

Furthermore, we found that a sustained $[Ca^{2+}]_i$ elevation occurred prior to the acrosome reaction if sperm were treated with ARIS plus asterosap. The sustained $[Ca^{2+}]_i$ elevation were highly susceptible to SKF96365 and Ni²⁺. In high-pH seawater, ARIS alone induced a sustained $[Ca^{2+}]_i$ increase which was similarly sensitive to SKF96365. Asterosap increased pH_i of sperm from 7.6 ± 0.1 to 7.7 ± 0.1. If the pH_i was artificially increased to 7.7 or higher, ARIS alone induced the acrosome reaction. Thus, we suggest that asterosap-induced pH_i increase is required to trigger the ARIS-induced sustained $[Ca^{2+}]_i$ elevation and the consequent acrosome reaction.

This thesis discusses the importance of studying the cooperative action on the sperm of multiple egg-coat components, like ARIS, asterosap and Co-ARIS in starfish, to understand the physiological process leading to the acrosome reaction and eventually to fertilization.