PARTIAL PURIFICATION OF SPERM-ACTIVATING AND SPERM-ATTRACTING SUBSTANCE IN THE ASCIDIAN, *CIONA INTESTINALIS*.

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Spermatozoa of the ascidian, *Ciona intestinalis*, exhibit chemotactic behavior to eggs prior to fertilization (Miller,1975; Yoshida and Morisawa, 1990). Here we tried to purify sperm-activating and sperm-attracting substance from unfertilized eggs. The eggs were suspended and incubated in artificial seawater for about 12 hr, and supernatant obtained by brief centrifuge (egg seawater) which exhibited strong sperm-activating and sperm-attracting activies was used as a starting material. The egg seawater was lyophilized, then extracted by absolute ethanol. After evaporation of ethanol, the extract was applied successively to reversed-phase (Sep-Pak C18), gel filtration (Bio Gel P-4), and cation exchange (CM-Sephadex C-25) column chromatographies. During these purification process, specific activity of sperm activation and attraction increased and both activities are always co-migrated. These suggest that sperm-activating and sperm-attracting substance is partially purified and both activities are derived from the same molecule. Nature of the substance is acidic, and molecular weight of this molecule was estimated by gel filtration as 2 ~ 2.2 kDa.

Role of Ca^{2+}-channel in activation of Xenopus egg by sperm extract.

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Potential changes as well as voltage-dependence on fertilization of amphibians are characteristics of sperm species, so that we attempted to obtain and characterize a sperm factor to induce egg activation. The extract obtained from Cynops pyrrhogaster sperm induced activation of dejellied, unfertilized Xenopus laevis eggs. The eggs treated with the extract elicited a short-lived, positive-going potential after appearance of a deep hyperpolarization. The pattern of potential changes induced by the sperm extract was quite similar to that by Cynops sperm, but not by homologous sperm. Immature oocytes or fertilized eggs show no potential changes upon treatment with the extract. When the unfertilized eggs were treated with the extract in 340 µM Ca^{2+}, the onset of cortical contraction and the positive-going potential was accelerated. The activation was inhibited by voltage-clamping at higher than -10 mV in 34 µM Ca^{2+}, or -20 mV in 340 µM Mg^{2+}, respectively. The activation was not inhibited at +20 mV in 340 µM Ca^{2+}. These results indicate that the activation by the sperm extract is mediated by opening of Ca^{2+} channels on egg plasma membrane.

PURIFICATION OF SPERM-ACTIVATING PROTEINS FROM UNFERTILIZED HERRING EGG. II.

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Unfertilized eggs of the pacific herring, Clupea palasii, release proteins which activate motility of herring spermatozoa. We have previously reported the purification of herring sperm activating proteins (HSAPs) and revealed that HSAPs are small acidic proteins on SDS-PAGE (Ohtake et al., 1990). To analyze the molecular natures of HSAPs, the HSAPs obtained from isoelectric focusing column were further analyzed by immobilized pH gradient gel electrophoresis. HSAPs are at least 5 proteins which pI values are 4.8, 4.9, 5.0, 5.1 and 5.4, respectively. The MW of all HSAPs purified on immobilized pH gradient gel electrophoresis are almost equal and are estimated less than 7 KD on SDS-PAGE. Gel filtration estimates the MW of HSAPs about 14 KD, which value is twice the MW obtained on SDS-PAGE. These suggest that HSAPs exist as dimers under the physilogical conditions. SDS-PAGE also revealed that HSAPs are composed of at least two peptides having different MW.

HEPARIN INHIBITS CALCIUM TRANSIENTS IN FERTILIZED SEA URCHIN EGGS INDUCED BY SPERM AND ITS SOLUBLE EXTRACTS.

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Fertilization is known to initiate a transient increase of intracellular calcium concentration (Ca_i-transient) and an accompanying change of membrane potential in sea urchin eggs.

Using aequorine, we previously showed that sperm and its soluble extract caused a Ca_i-transient in fertilized eggs from which fertilization membranes and the hyaline layers were removed.

In this report, we quantitatively measured intracellular Ca^{2+} concentration with Indo-1 microfluorometry and showed that the microinjection of heparin (final concentration of approx. 1mg/ml) into fertilized eggs inhibited the Ca_i- and voltage- transients by sperm and sperm extract. The sperm extract which was diluted to 10-fold with artificial sea water (ASW) increased the intracellular Ca^{2+} concentration from 240nM to 760nM (in average values). The sperm extract which diluted to 1000-fold lost activity. The molecular weight of the active factor in the sperm extract was less than 5000.