ALTERATION OF CELLULAR EVENTS INVOLVED IN THE FIRST CLEAVAGE OF SEA URCHIN EGG BY TWO MARINE TOXINS : "MAITOTOXIN" AND "CRASSOLIDE"

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In this study the sea urchin egg model was used to investigate the effects of **maitotoxin** (**MTX**) synthetized by the epiphytic dinoflagellate ***Gambierdiscus toxicus*** (1) and by **crassolide** a diterpene isolated from the Indonesian soft coral ***Lobophytum crassum*** (13).

Sea urchin eggs are convenient to use for bioassays since it is easy to obtain cells dividing synchronously. In addition, the schedule of ionic events along the cell cycle is one of the best known showing the same succession as mammal cells in culture (2, 3, 4, 5, 8, 14).

Maitotoxin is known to stimulate a large spectrum of calcium dependent physiological processes ; it is a potent activator of calcium influx in a large variety of cells (6, 7, 11, 12). Fertilization and cleavage of sea urchin eggs were inhibited in a dose dependent manner by **MTX** (fig. 1, 2). This toxin increased the permeability to Ca^{2+} of both plasma and intracellular membranes and modified K^+ and Na^+ distribution in the female gametes (fig. 3, 4, 5, 6). Toxin induced changes in ion permeabilities were observed at a concentration much higher than those inhibiting fertilization and did not evolve rapidly. Therefore, the blockage of fertilization which occured at low MTX concentrations and appeared in a short time is probably not due to ion transport perturbation : a modification of the unfertilized egg plasma membrane by the hydrophylic toxin could be involved (9).

Crassolide (fig. 7) inhibited the cell cleavage of sea urchin eggs without affecting fertilization. The effect was observed at concentrations above 2×10^{-5} M in egg suspensions (fig. 8). Addition of crassolide between 5 to 40 minutes after fertilization totally blocked the first cleavage (fig. 9). When added between 50 to 60 min. post fertilization, crassolide produced polynucleated cells in embryos. Moreover, it did not affect egg permeability to Na^+ and Ca^{2+} but caused a 0.2 unit increase of intracellular pH of fertilized eggs coupled with a proton efflux (fig. 10). Crassolide which affects neither Ca^{2+} influx nor Ca^{2+} permeability of reticular store could be used as negative control when analysing calcium changes in short -term toxicological studies. A possible relationship between pH increase and cell cleavage needs further investigations.

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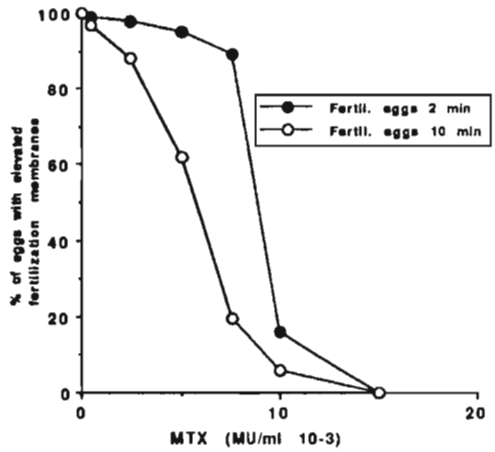

Fig. 1 : Effect of increasing concentration of MTX on fertilization of sea urchin eggs

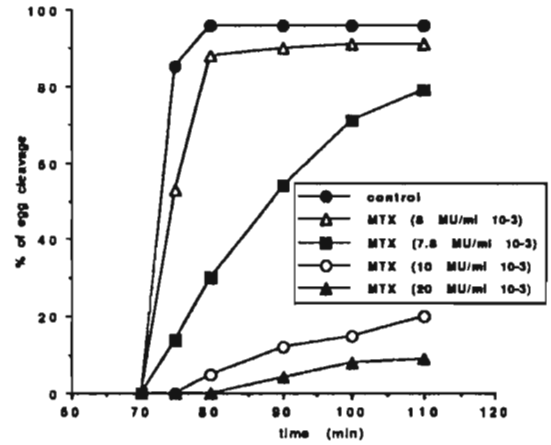

Fig. 2 : Effect of increasing concentration of MTX on sea urchin egg cleavage. Toxin was added to egg suspension 30 sec. after insemination.

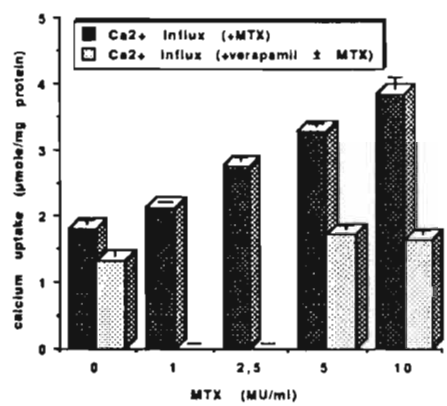

Fig. 3 : Effect of increasing concentration of MTX on Ca2+ influx into unfertilized sea urchin eggs

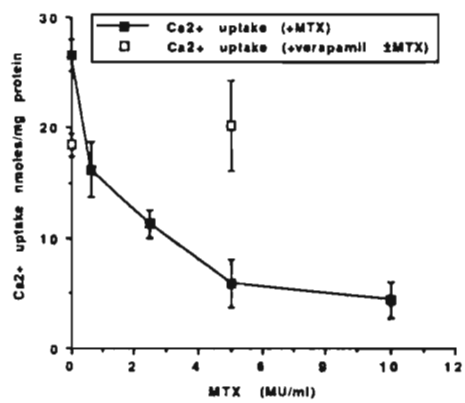

Fig. 4 : MTX reduced the rate of ATP-dependent 45Ca sequestration by isolated cortices

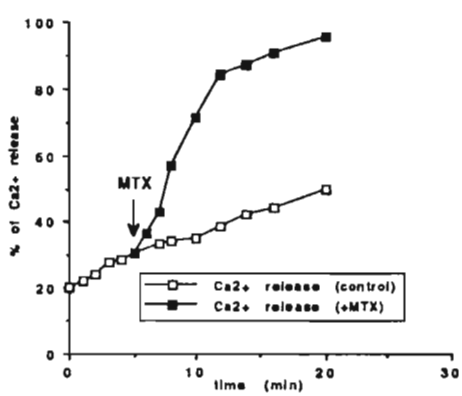

Fig. 5 : MTX induced a release of Ca2+ in preloaded isolated cortices

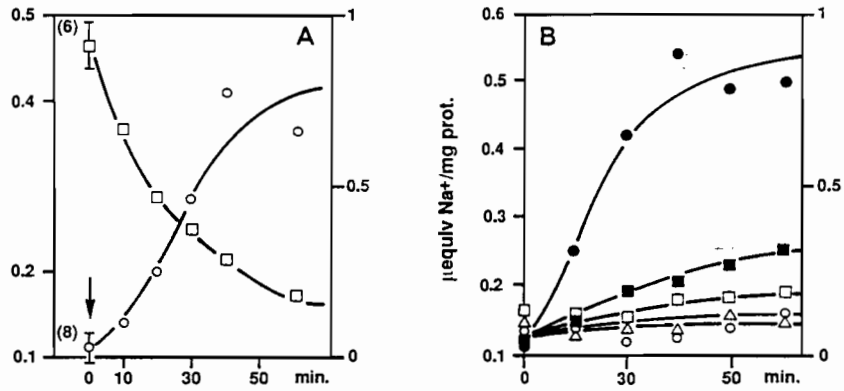

Figure 6 : effect of MTX on Na^+ and K^+ intracellular contents of unfertilized sea urchin eggs.

Toxin was present in egg suspension in which samples were taken at appropriate times for measurement of ion content

A : effect of 0.75 MU/ml MTX on Na^+(O) and K^+ (□)

B : dose-response of MTX on Na^+ content ; Control (O), MTX : 0.75 10^{-3}MU/ml (Δ), 7.5 10^{-3}MU/ml (□), 75 10^{-3}MU/ml (■), 0.75 MU/ml (●)

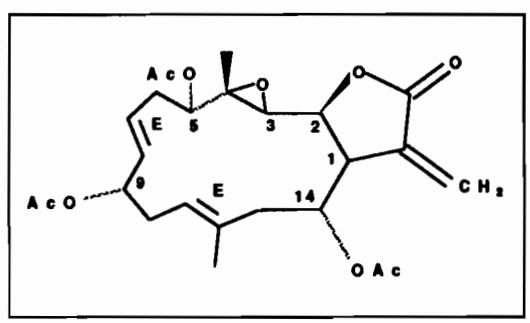

Fig. 7 : CRASSOLIDE

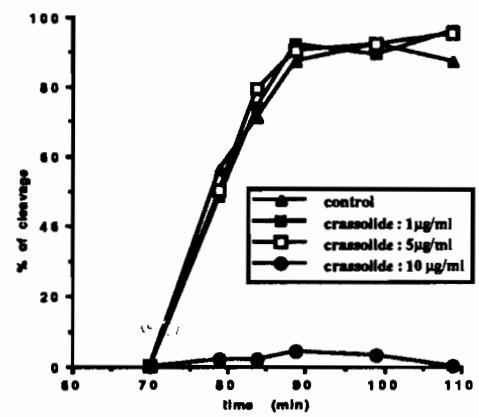

Fig. 8 : Effect of increasing concentrations of crassolide on sea urchin egg first cleavage. Toxin was added 30 s. after insemination.

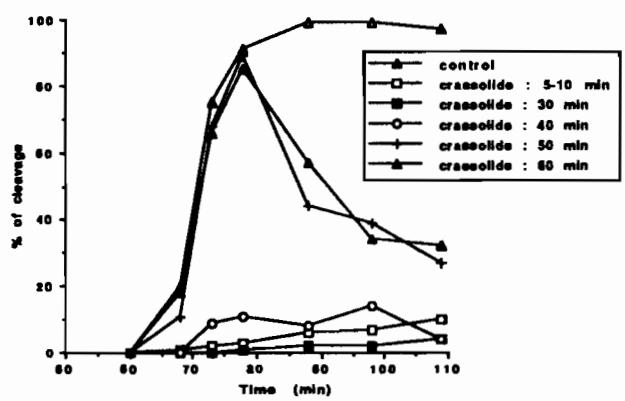

Fig. 9 : Effect of crassolide on the rate of cleavage of sea urchin eggs depending on post-fertilization times of crassolide addition

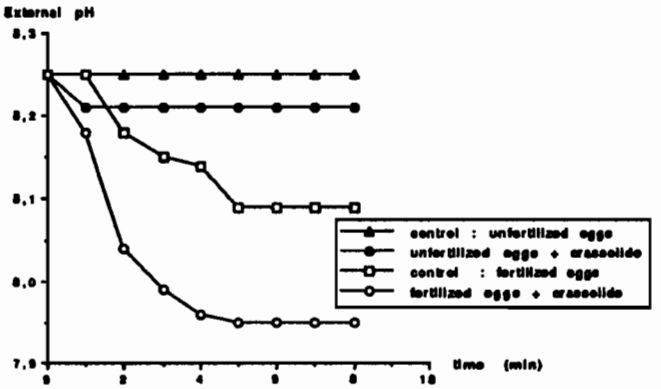

Fig. 10 : Time-course of external pH following ciguatoxin addition (50µg/ml) to sea urchin eggs

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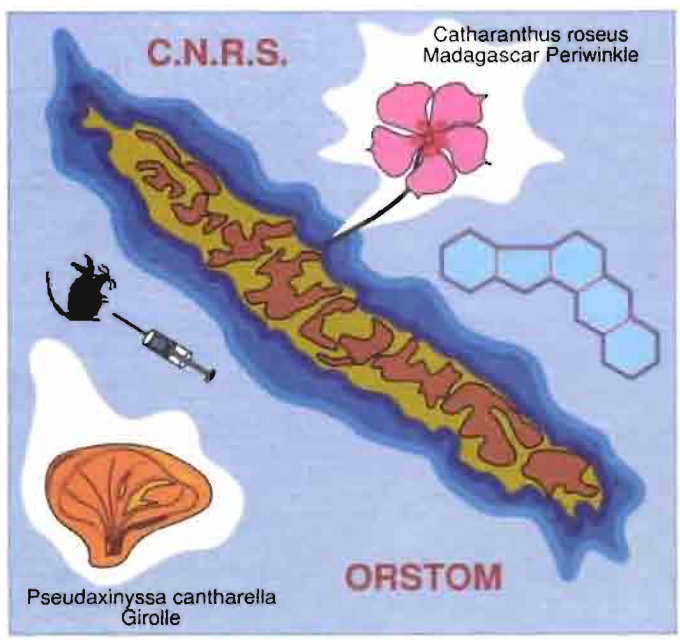

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