PERIODIC CHANGE IN THE CONTENT OF ADENOSINE 3'5'-CYCLIC MONOPHOSPHATE WITH CLOSE RELATION TO THE CYCLE OF CLEAVAGE IN THE SEA URCHIN EGG

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SUMMARY: The content of adenosine 3'5'- cyclic monophosphate (cAMP) in sea urchin eggs, Hemicentrotus pulcherrimus, increased gradually after fertilization to about 10-fold that in unfertilized egg, and decreased rapidly during cytokinesis of the egg to the level found in unfertilized egg. The same profile of the change in cAMP content as found during first cleavage, was also observed during second and third cleavage. The periodic change in cAMP content in the sea urchin egg seems to be repeated with close relation to the cycle of cytokinesis.

INTRODUCTION: Since Sutherland and Rall (1960) have reported the role of cAMP as an intracellular mediator in glycolysis, this nucleotide has been demonstrated to mediate a remarkable array of physiological phenomena (Robison, Butcher and Sutherland 1968). As adenyl cyclase in the sea urchin egg has been demonstrated to be activated and the content of cAMP has been reported to increase upon fertilization (Nath and Rebhun 1973), this nucleotide might be expected to take a part in the stimulation of glycogenolysis, which is activated at fertilization (Yasumasu et al 1973), and other cell functions fertilization.

In the present paper, we report an increase in the content of cAMP in the sea urchin egg upon fertilization, and a periodic change in the level of this nucleotide with close

relation to the cycle of cleavage in the sea urchin egg after fertilization.

MATERIAL AND METHODS: The material used was the eggs and sperm of the sea urchin Hemicentrotus pulcherrimus. The eggs were inseminated and allowed to develop at 22°C. A part of the fertilized egg suspension was taken off every 5 minutes, and eggs were collected by centrifugation at 5,000 rpm for 2 minutes on a refrigerating centrifugator. The egg pellet thus obtained was frozen with liquid nitrogen, then homogenized in a mortar with an equal volume of 10 % perchloric acid in the presence of liquid nitrogen. The supernatant, obtained after centrifugation of the homogenate at 5,000xg for 15 minutes, was neutralized in an ice bath with saturated K_2CO_3, and then centrifuged again at 100,000xg for 1 hr. The supernatant thus obtained was filtered through a millipore filter. Two ml of mixture containing 40 mM glycylglycine buffer at pH 7.2, 10 mM $MgCl_2$ and appropriate concentration of the filtered supernatant in a quartz cuvette was placed on a two wave length, dual beam spectrophotometer, model Hitachi 356. The content of cAMP was measured enzymatically on the spectrophotometer at 30°C by recording (recorder model Hitachi QPD 36) the decrease in the difference of the absorbancies between 260 nm and 300 nm produced by the coupled reaction of adenosine deaminase and alkaline phosphatase following the action of phosphodiesterase (Drummond and Perrotte-Yee 1961). The minimum level of cAMP which could be measured with this metnod was about 30 pmoles.

The enzymes used were purchased commercially from Boeringer Mannheim Co. West Germany.

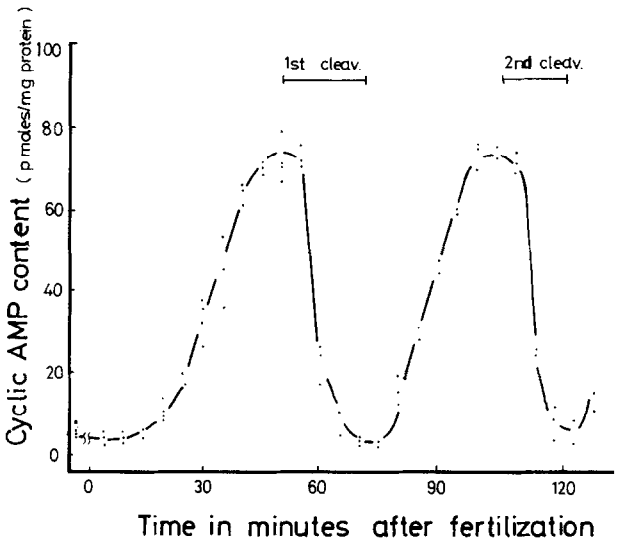

Fig 1. Change in the content of cAMP in the sea urchin egg after fertilization

RESULTS AND DISCUSSION: As shown in Fig 1, the content of cAMP in the unfertilized egg was about 5 pmoles per mg protein, equivalent to the concentration of 10^{-7}M or less in the egg. It began to increase 15 minutes after insemination and reached a concentration of 1.4×10^{-6}M (about 70 pmoles per mg protein) at 55 minutes after fertilization, where the first cleavage was going to take place.

The increase in cAMP in the fertilized egg may result from activation of adenyl cyclase upon fertilization, as demonstrated by Castaneda and Tyler (1968). Thereafter, cAMP content was found to decrease rapidly below its level in the unfertilized egg within 15 minutes after reaching its maximum level. The first cleavage of the egg occurred within this period. During second cleavage of the sea urchin egg, the same profile of change in the cAMP level was observed. Since cAMP level was 67.6 ± 9.4 pmoles per mg protein at the begining of the third cleavage and was 4.1 ± 1.6 pmoles per mg protein

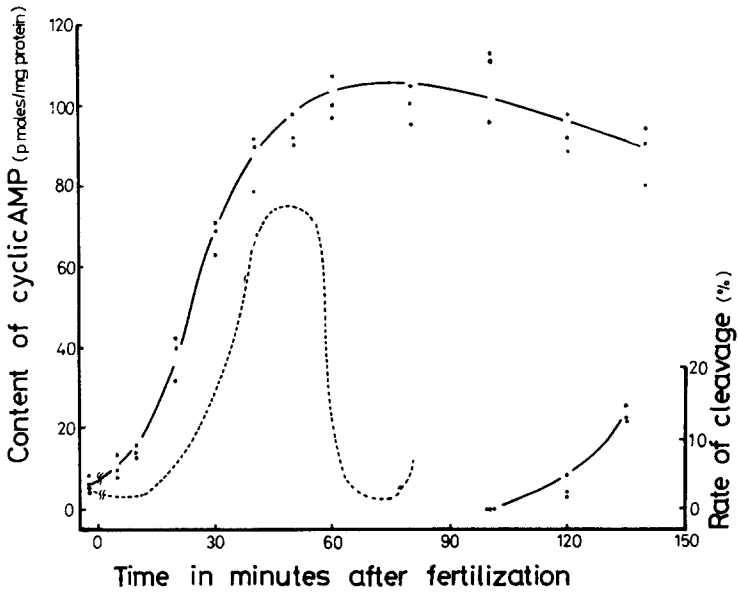

Fig 2. The effect of caffeine on the content of cAMP in the sea urchin eggs. Dotted line shows the change in cAMP content in the sea urchin egg without caffeine.

at the end of the third cleavage, the same change in its content may be observed during the third cleavage of the egg. Therefore, it appears that the level of cAMP in the eggs changes periodically with close relation to the cycle of the cell division. The relation of the decrease in cAMP level to mitosis of cultured cell has been also demonstrated by Burger et al (1972). It might be also supposed that the periodic change in the content of cAMP would be due to the change in the activity of phosphodiesterase in the sea urchin egg, since the activity of adenyl cyclase remains at a high level after activation by fertilization (Castaneda and Tyler 1968).

Administration of 10^{-3}M caffeine (which is an inhibitor of phosphodiesterase) to the fertilized egg caused an increase in the rate of cAMP accumulation (Fig 2). Extraordinarily

high levels of cAMP were observed and the periodic change in the content of cAMP in fertilized egg were not detectable following caffeine administration.

These facts suggest a role for phosphodiesterase in the periodic change in cAMP content of sea urchin eggs. Moreover, the consumption of cAMP, observed during the cleavage of the sea urchin egg, may be closely related to cell division since caffeine was found to inhibit both cell cleavage and the decrease in cAMP.

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