INTRACELLULAR CALCIUM AND CELL CLEAVAGE IN EARLY EMBRYOS OF *XENOPUS LAEVIS*

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INTRODUCTION

There is growing evidence that cell cleavage involves contraction in the region of the furrow and relaxation above the most distant parts of the spindle (see references 15 and 16). The contractile mechanism has been likened to that of muscle, mainly as a result of studies of glycerinated models of dividing cells (7, 9) and extraction of an actomyosin-like material (8, 11).

Muscular contraction is activated by calcium ions, and various workers have implicated the Ca ion as a regulator of cell cleavage (15, 16). We now report experiments designed to test this hypothesis. The results show that Ca ions are essential for cleavage; but, at this stage, it is not possible to decide whether or not *changes* in the level of ionized Ca regulate cleavage as opposed to cleavage merely having a requirement for some ionized calcium.

MATERIALS AND METHODS

Fertilized eggs were obtained from pairs of mature *Xenopus laevis* by injection of chorionic gonadotrophin. The eggs were routinely kept in Holtfreter's solution. The bulk of the jelly was removed from the eggs by treatment for about 5 min with a mixture of 2% cysteine and 0.1% papain at pH 8 (4). This treatment also seemed to soften the vitelline membrane, making it easier to microinject substances into the eggs. Subsequent development was normal.

Substances were pressure-injected into the eggs through micropipettes of tip diameter 10–15 μ. The pipettes were drawn with a glass capillary inside to assist filling from the shank. Pipettes of this diameter were sufficiently large to allow easy injection but small enough for the egg to heal satisfactorily after withdrawal of the pipette. Eggs were held for injection in small depressions in a wax-coated Petri dish. Injections were normally made into one blastomere soon after completion of first cleavage, the other blastomere serving as a control. About 0.1–0.2 μl was normally injected into about 0.7 μl. Control injections of up to 0.5 μl of buffered isotonic KCl were without effect on either the timing or the microscopic appearance of cleavage.

The main substances injected were KCl, Ca-buffers which are mixtures of Ca and ethylene glycol *bis* (β-aminoethyl ether) *N*, *N'*-tetraacetic acid (EGTA) (see references 2, 3, and 10), and the calcium-sensitive protein, aequorin (1, 2, 3, 14). All the solutions were close to pH 7.2. Ca-EGTA buffers were normally 100 mM with respect to EGTA: the resulting intracellular concentration should therefore have been between 10 and 20 mM. This rather high concentration was used because preliminary measurements showed that *Xenopus* eggs contained about 15 mM total calcium. Nothing is known about the location or state of this rather large amount of Ca, but it may be associated with the yolk.

The behavior of eggs injected with KCl or Ca-EGTA buffers was normally followed microscopically for several hours. Some material was also fixed in either Bouin's or Smith's fixative, cut into serial sections, and stained with hemalum and eosin. The light emitted from aequorin-injected eggs was monitored by mounting individual eggs over a sensitive photomultiplier (EMI type 9635B) attached to a pen-recorder. Both the sample of aequorin and the recording circuit were the same as those used by Baker, Hodgkin, and Ridgway (1971). At the end of each experiment the eggs were lysed in 90 mM Ca:100 mM EGTA to assess how much aequorin was left.

Measurements of electrical coupling between the blastomeres were made in the standard way with two microelectrodes (5).

Toad eggs have been used for the present experiments because of their large size, but they may not be a particularly good system for studying cleavage because in amphibian eggs much of the membrane in the cleavage furrow seems to be formed *de novo* and separation of the two daughter cells is not achieved simply by stretching the existing surface (12, 13). Despite the technical difficulties, it would seem worth attempting similar experiments with Ca-EGTA buffers in sea urchin eggs.

RESULTS AND DISCUSSION

Experiments with Ca-EGTA Buffers

Injection of the Ca-chelating agent EGTA produced marked relaxation of the egg cortex especially around the injector and often led to swelling and bursting of the cell. In those EGTA-injected eggs which did not burst, subsequent cleavage failed to occur. These observations are in striking

TABLE I

Effect on Cleavage of Injecting Ca-EGTA Buffers

The ionized Ca concentration has been calculated on the assumption that intracellular pH = 7.1 and ionized Mg = 20 mM. Temperature: 20°C; *n* is the number of embryos examined.

Ca:EGTA ratio	Estimated ionized Ca (M)	*n*	%			
			Blocked	Slowed	Normal	Cleaving
0–100	—	13	100	—	—	—
5–100	2×10^{-8}	19	79	21	—	21
10–100	4×10^{-8}	26	65	35	—	35
20–100	8×10^{-8}	19	31.5	37	31.5	68.5
45–100	3×10^{-7}	18	17	28	55	83
90–100	3×10^{-6}	15	—	—	100	100

contrast to those made after injections of KCl which had no immediate or delayed effects on the appearance of the cell or on cleavage.

Table I and Fig. 1 summarize data for a series of Ca-EGTA buffers. The following points should be noted:

(*a*) In the presence of adequate Ca (90 mM Ca: 100 mM EGTA), EGTA does not affect cleavage. It seems, therefore, that the observed effects of EGTA alone are the result of Ca chelation rather than a toxic action of EGTA.

(*b*) EGTA containing a small amount of Ca either blocks or slows the onset of cleavage. Buffers containing 10 mM Ca and 100 mM EGTA produce little or no cortical relaxation and swelling, but affect cleavage. Slowing of cleavage seemed to be associated with an increase in the length of the division cycle.

(*c*) The effects on cleavage are restricted to the injected blastomere. Both blastomeres were affected only when the injection was performed before the end of first cleavage. The uninjected blastomere continues to cleave, the cells becoming progressively smaller, whereas the injected blastomere remains uncleaved at its initial size.

In view of the fact that the blastomeres of *Xenopus* embryos are coupled electrically, it is surprising that the effects of EGTA are restricted to the injected blastomere. Tests showed that the EGTA-injected cells remained coupled, suggesting that the low-resistance junctions in this tissue must be relatively impermeable to EGTA. The data do not provide any information on the permeability of the junction to Ca.

(*d*) Examination of fixed material shows a number of nuclei in the uncleaved blastomere. Although it is possible that some form of nuclear fragmentation has occurred, these observations suggest that nuclear division can take place at a lower ionized Ca concentration than can cytoplasmic cleavage.

(*e*) Immersion of embryos in Ca-free medium containing EGTA had no effect on cleavage.

In conclusion, these experiments with Ca-EGTA buffers show that cytoplasmic cleavage requires a low intracellular concentration of ionized calcium. They also show that even lower intracellular concentrations of Ca result in relaxation of the egg cortex (see reference 6). The absolute levels of ionized Ca producing these effects are difficult to specify accurately because the ionic Ca stabilized by a particular Ca-EGTA buffer depends on both the pH and the concentration of ionized Mg inside the cell, neither of which is known for the *Xenopus* egg. The values given in Table I are estimates, but

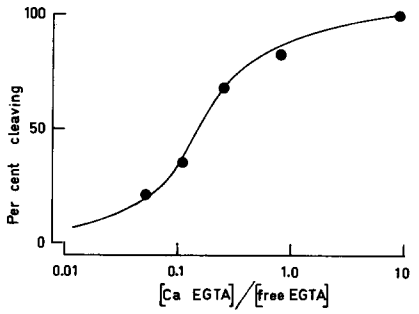

FIGURE 1 Intracellular concentration of ionized Ca and cell cleavage. Temperature: 20°C. Abscissa: ratio of [Ca EGTA]/[free EGTA] which is proportional to the concentration of ionized Ca. Note the log scale Ordinate: percentage of injected cells undergoing cleavage.

as such can be compared with data for large nerve and muscle fibers—the only other cells in which the intracellular ionized Ca concentration is known at all accurately. The ionized Ca concentration in squid axons is about 3×10^{-7} M (2), and crab muscle contracts when the ionized Ca concentration is raised to about 10^{-6} M (10). These concentrations are strikingly similar to the threshold Ca concentration for cleavage in *Xenopus* eggs.

The data suggest that cleavage requires a Ca concentration similar to that found inside other cells, but they do not enable a distinction to be made between the possibility that a relatively low *constant* concentration of ionized Ca is a necessary cofactor for cleavage and the possibility that *changes* in ionized Ca serve to initiate and control cleavage, much as changes in Ca regulate muscular contraction.

Experiments with Aequorin

In an attempt to see whether the intracellular concentration of ionized Ca changes during division, we injected the Ca-sensitive protein aequorin (14) into a number of *Xenopus* embryos—either before or just after first cleavage.

Although injection proved rather difficult, sufficient aequorin was injected into eight cells to enable measurements of light emission during the division cycle. Five of these eight cells showed an increase in light output at the time of both first and second cleavages. These transient changes were seen in eggs immersed in Ca-free media containing EGTA, which suggests that they could not have resulted from changes in Ca entry during cleavage.

These experiments should be treated with considerable caution. *Xenopus* eggs are very opaque and much of the light produced by the aequorin reaction is probably absorbed within the egg. A small reduction, during cleavage, in this internal absorption could account for the observed changes. A further complication is that we were unable to change the light output by injection of Ca-EGTA buffers, which suggests that the aequorin must be sequestered into some compartment where it is unavailable to the injected EGTA.

CONCLUSIONS

The experiments with Ca-EGTA buffers seem to provide clear evidence that cell cleavage requires a definite but low concentration of ionized Ca. The experiments with aequorin suggest that the average concentration of ionized Ca in the egg may increase at the time of cleavage; but these observations must be treated with caution, and it is not yet clear whether cleavage merely requires the presence of some Ca or whether it is regulated by local changes in the concentration of ionized Ca.

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