Calcium Buffer Injections Block Cytokinesis in *Xenopus* Eggs

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Vincent *et al.* (1) reported that injections of EGTA giving final cytosolic concentrations of about 8 m*M* had no detectable effects on cytokinesis; but final concentrations of about 30 m*M* delayed first division in half the eggs, while blocking second division in all injected eggs. [We have doubled the values given by Vincent *et al.* because they assume an accessible volume that is half that of the whole *Xenopus* egg; the true figure seems close to a quarter (2, 3)]. Inhibition at such extremely high buffer concentrations might well suggest that calcium is **not** involved in the natural control of cytokinesis in *Xenopus* eggs. However, the results of our study of buffer inhibition in fucoid eggs (4) suggested a reinvestigation with another buffer more closely matched to the calcium level in the cytosolic region responsible for initiating and driving the division process (5). Table I shows the results of injecting 5,5'-dibromo-BAPTA, a buffer with a cytosolic K_D of about 5 μM. The lower figure in each range corrects for total accessible volume, on the assumption that about 20% of the egg volume is accessible to the injected buffer; buffer accessibility was calculated, in turn by taking 70% of the egg volume as inaccessible yolk (2) and about 70% of the cytosol as water (3). The higher figure in each range takes into account the likelihood that, at the time of action, the buffer had diffused out through a sphere only about 800 μm in diameter instead of filling the whole 1200 μm diameter egg.

This buffer is about one hundred times more effective than EGTA in delaying or blocking cytokinesis. Detectable delays occur in response to final cytosolic buffer concentrations as low as 0.1 m*M*. This finding supports both the hypothesis that calcium controls cytokinesis, and the validity of the following equation (4) in *Xenopus* eggs:

$$B = rC \cdot (D_{Ca}/D_B) \cdot [(1 + f)^2/f]$$

where: B is the total concentration of buffer within the cytosol needed to facilitate calcium diffusion r-fold; C is the concentration of free calcium within the biologically effective, dynamically maintained high calcium zone; D_{Ca} and D_B are rough values for the cytosolic diffusion constants (640 and 38 μ^2/s) of free calcium and of BAPTA-type calcium buffers respectively; $f = K_D/C$, where K_D is the calcium dissociation constant of the particular buffer employed.

Further support for these points emerges from two further quantitative tests. First, the total calcium found in *Xenopus* eggs during early cleavages is about 8 m-mole/liter egg (6), which about equals the 7.5 m-mole/liter egg of EGTA in the highest dose injected by Vincent *et al.* This rough equivalence is consistent with our assumption that a combination of release from internal reserves, plus some entry from the medium, provided enough calcium to neutralize the EGTA injected by Vincent *et al.* Second, the observed inhibitory buffer concentrations are consistent with the above equation if some quite reasonable assumptions are made. Specifically, if one assumes that doubling the effective diffusion constant of calcium will suffice to barely inhibit cytokinesis (*i.e.*, r = 1), that the calcium in the "target" zone or C is 5 μM, that D_{Ca}/D_B for dibromo-BAPTA is 17 (4), and for the smaller EGTA molecule about 10, and that K_D for EGTA is about 0.01 μM (7), **then** the equation predicts just inhibitory levels of dibromo-BAPTA and of EGTA of 0.4 and 30 m*M*, respectively.

Table I

Inhibition of Xenopus *egg cleavage by the injection of 5,5'-dibromo-BAPTA calcium buffer*

Buffer conc. (m*M*)	n	Avg. first cleavage delay (minutes)	Death (hours)
2.0–0.6	40	180	≈6
0.76–3.3	69	100	≤24
0.23–1.4	40	25	None
0.10–0.45	120	7	None
0.007–0.24	50	No effect	None

Injection at 40 to 100 min after insemination. Done at 15°C, at which first cleavage begins in uninjected controls about 3 h after fertilization. Eggs were co-injected with 0.1 mol of Ca^{2+} per mol of buffer ($K_d = 3.6 \times 10^{-6}$ *M*).

In a second series of experiments, we delayed buffer injection until furrow initiation had clearly begun at the animal pole. The cytosolic concentration range of buffer (based on the same assumptions as above) was 1.5–5.1 m*M*. In 15 cases, we consistently arrested the first cleavage furrow as it progressed across the animal hemisphere. This suggests that the buffer may be delocalizing an elevated calcium region directly involved in the furrowing process, as opposed to indirectly inhibiting cytokinesis through some earlier calcium-dependent event occurring during the cell cycle.

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