Reduced External Calcium or Sodium Stimulates Calcium Influx in \textit{Pelvetia} Eggs

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\textbf{Abstract.} The effect of external calcium and sodium ion concentrations on the calcium fluxes on the \textit{Pelvetia fastigiata} De Toni egg was measured. Decreasing external [Ca\textsuperscript{2+}] greatly increased the permeability of the eggs to Ca\textsuperscript{2+}; at 1 mM external Ca\textsuperscript{2+} this permeability was 60 times as great as it was at the normal [Ca\textsuperscript{2+}] of 10 mM. Lowering the external [Na\textsuperscript{+}] also increased Ca\textsuperscript{2+} influx; at 2 mM Na\textsuperscript{+}, the Ca\textsuperscript{2+} influx was 2-3 times as great as it was at the normal [Na\textsuperscript{+}] if choline was used as a Na\textsuperscript{+} substitute. Lithium was less effective as a Na\textsuperscript{+} substitute in increasing Ca\textsuperscript{2+} influx. The extra Ca\textsuperscript{2+} influx in low [Na\textsuperscript{+}] seemed to be dependent on internal [Na\textsuperscript{+}]. The Ca\textsuperscript{2+} efflux increased transiently and then declined in low Na\textsuperscript{+} media.

\textbf{Key words:} Ca\textsuperscript{2+} flux -- Na\textsuperscript{+} -- Ca\textsuperscript{2+} interactions -- Ion fluxes -- \textit{Pelvetia}.

\textbf{Introduction}

In a previous paper (Robinson and Jaffé, 1975) it was shown that one of the first responses of the radially symmetric egg of the brown alga, \textit{Pelvetia fastigiata} De Toni, to unilateral light is to drive a calcium current through itself. It was suggested that the function of this current was the production of an intracellular Ca\textsuperscript{2+} gradient which acted as an essential link between the external light signal and the ultimate response of the zygote: the formation of a rhizoidal bulge at the darker end where more Ca\textsuperscript{2+} enters. If this idea is correct, it should be possible to mimic the polarizing effect of unilateral light by putting the cells in an external gradient such that more Ca\textsuperscript{2+} is forced in one end than the other (assuming that the efflux is unchanged).

The problem then is to find substances which will affect calcium influx in as specific a way as possible. Obviously, one such agent might be calcium itself. Since its entry is presumably largely a passive process, increasing the external calcium concentration ([Ca\textsuperscript{2+}]\textsubscript{o}) would be expected to increase Ca influx while decreasing [Ca\textsuperscript{2+}]\textsubscript{o} would decrease influx. A second candidate for the calcium flux modifier is sodium. Sodium competes with calcium for entry into a number of cells, including vertebrate and invertebrate nerve and muscle (for a review, see Blaustein, 1974), and lowering [Na\textsuperscript{+}]\textsubscript{o} increases calcium influx in these cells. In several of these same cells, lowering [Na\textsuperscript{+}]\textsubscript{o} also reduces calcium efflux by inhibiting Na-Ca exchange. In view of these two effects of lowering [Na\textsuperscript{+}]\textsubscript{o}, a gradient of sodium concentration should be a very effective agent for producing intracellular calcium gradients if the \textit{Pelvetia} membrane behaves as do the membranes of nerve and muscle cells.

It was with this purpose that I began to investigate the effects of [Ca\textsuperscript{2+}]\textsubscript{o} and [Na\textsuperscript{+}]\textsubscript{o} on calcium fluxes in \textit{Pelvetia} zygotes. These measurements were done primarily on eggs that were 6-8 h after fertilization. At this stage, the eggs are still single-celled and have not yet irreversibly formed a rhizoid-thallus axis. Further, it is at this stage that the endogenous calcium current is greatest.

\textbf{Material and Methods}

\textbf{Influx Experiments}

Fertilized \textit{Pelvetia fastigiata} eggs (90 \textmu m in diameter) were obtained as described in Jaffé and Neuscheler (1969). The eggs, which glue themselves tightly to almost any substratum by 5 h after fertilization, were uniformly distributed in 3.2-cm diameter Pyrex planchetts which fitted into a Nuclear-Chicago gas-flow counter. 20,000 to 30,000 eggs were added to each planchet in 2 ml of artificial sea water (ASW). After the eggs were stuck to the planchetts, most of the ASW was removed and the planchetts were placed in the
appropriate medium containing $^{45}\text{Ca}^{2+}$ (usually 0.5 mCi $^{45}\text{Ca}$/mmol Ca). After exposure to $^{45}\text{Ca}^{2+}$, the planchets and eggs were washed in natural sea water for 20-30 min, dried, and the $^{45}\text{Ca}$ counted. The lengthy wash is necessary to remove $^{45}\text{Ca}^{2+}$ from the cell wall (Robinson and Jaffe, 1973). Standards were prepared by adding a measured amount of $^{45}\text{Ca}$-ASW to egg-containing planchets.

**Efflux Experiments**

Freshly shed eggs were put in $^{45}\text{Ca}$-ASW and concentrated to a dense suspension by allowing them to settle and removing the supernatant. They were then drawn into a 12-cm length of glass tubing, 2 mm inner diameter. The ends of the tube were capped with sealed plastic tubing, the tube was mounted horizontally, attached to a motor, and rotated about its axis at 3 rpm. As the egg became sticky, they adhered to the walls of the tube, forming a uniform egg cylinder. Unlabeled ASW was then passed through the tube at about 1 ml/min and, after all extracellular $^{45}\text{Ca}^{2+}$ was removed, the effluent during 5-min intervals was collected in scintillation vials. Scintillation fluid was then added to the vials and the radioactivity was counted in a Beckman LS-230 liquid scintillation spectrometer. The particular advantage of this method is the small volume of the tube (0.4 ml), which made it possible to change the medium rapidly while keeping the volume of effluent small.

**Media**

All solutions were variations of a standard artificial sea water of the following composition: NaCl, 421 mM; MgCl$_2$, 50 mM; Na$_2$SO$_4$, 28 mM; CaCl$_2$, 10 mM; KCl, 10 mM; NaHCO$_3$, 2 mM; pH 8.0.

In low-sodium media, either LiCl or choline chloride was substituted isosmotically for NaCl. The choline Cl was recrystallized from ethanol to remove impurities. Since the membranes of *Pelvetia* eggs respond to decreases in osmolarity by releasing ions (Nuccitelli and Jaffe, 1976b) all solutions in a given experiment were adjusted to within 1% of the same osmolarity, using an Advanced Instruments (Needham Heights, Mass., USA) Hi Precision Osmometer 3R.

When the calcium concentration was varied, magnesium was added or subtracted from the solution so that the sum $[\text{Ca}^{2+}] + [\text{Mg}^{2+}]$ remained constant at 60 mM. The specific activity of the $^{45}\text{Ca}^{2+}$ in the solutions of different $[\text{Ca}^{2+}]$ was the same in a given experiment.

**Results**

**Calcium-dependent Calcium Influx**

The data on calcium influx in Figure 1 were obtained from a single batch of *Pelvetia* eggs. At 6 h after fertilization the eggs were placed in $^{45}\text{Ca}^{2+}$ media containing different concentrations of calcium, and samples were removed at 5, 15 and 45 min. The right-hand axis shows the number of counts per minute per egg divided by the number of counts per minute per picomole of calcium in the extracellular media. Using the 5-min points of the curves and the area of the eggs ($2.5 \times 10^{-4}$ cm$^2$), the influx at each concentration can be calculated. These fluxes are shown in Figure 2, expressed as a fraction of the influx at 10 mM Ca. Also shown in that figure is the ratio of the calcium permeability at the various $\text{Ca}^{2+}$ concentrations to the permeability at 10 mM $\text{Ca}^{2+}$. These ratios were calculated by dividing the influx ratios by the corresponding concentration ratios. These calculations neglect any effect of the small changes in the membrane potential resulting from changing $[\text{Ca}^{2+}]_0$ (Weisenseel and Jaffe, 1972). Since the permeability ratios form a fairly simple curve, this curve was used to calculate the flux ratios at...