CALCIUM ION REGULATION OF FLAGELLAR BEAT SYMMETRY IN REACTIVATED SEA URCHIN SPERMATOZOA

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SUMMARY: Spermatozoa extracted with Triton X-100 solutions containing 0.005 M $CaCl_2$ have nearly symmetrical flagellar beat patterns and straight swimming paths when reactivated in ATP solutions containing low concentrations (10^{-9} M) of free calcium ion. Increased calcium in the reactivation solutions, or omission of calcium from the extraction solution, causes asymmetrical flagellar beating, and circular swimming paths.

Following removal of the flagellar membrane by 50% glycerol (2,11) or detergents (8), the movements of sea urchin spermatozoa can be reactivated by ATP. These preparations are of great value for experimental study of flagellar movement (4-6,9), especially since development of membrane removal procedures using Triton X-100, which lead to nearly 100% reactivation of movement (8). The Triton procedure was originally developed by Gibbons and Gibbons for spermatozoa of the Hawaiian sea urchin, Colobocentratus atratus, but they also used the same procedure to successfully reactivate spermatozoa from the Pacific Coast sea urchin, Strongylocentrotus purpuratus (10). In attempting to apply the Triton procedure to spermatozoa from S. purpuratus in our laboratory, we found that some aspects of the procedure used by Gibbons and Gibbons were unexpectedly critical. Subsequent work indicated that consistent results depended upon careful control of the calcium ion concentrations in both the extraction and reactivation solutions. An effect of calcium had been noted previously in experiments with tritonated Colobocentratus spermatozoa extracted with 0.5 M KCl to remove dynein (9).

We obtained quantitative data on the effects of calcium by photographing the swimming paths of reactivated spermatozoa, and measuring the curvature of the paths. A similar photographic method was used by Gibbons and

Gibbons (8), but we have expressed our results in different form, as a turning rate, in radians per beat. Spermatozoa were photographed while swimming at the upper surface of an open drop of reactivation solution on a microscope slide, using a 16 X objective and a total magnification on 35 mm film of 64 X. Multiple exposures with flashes at 25 Hz or 12.5 Hz were used to record the sperm paths. The finished films were projected, and the angular change in direction of each spermatozoon over a series of flashes was measured with a protractor. These values were then converted to radians per beat, by using the mean beat frequency of 25 Hz which was typical of the reactivated spermatozoa at the ATP concentration used in these experiments. We found no effects of calcium on the beat frequency of ATP-reactivated spermatozoa.

Our procedures for extraction and reactivation of spermatozoa from the sea urchin, Strongylocentrotus purpuratus, are based on those of Gibbons and Gibbons (8), with one important modification. Concentrated spermatozoa, collected from the aboral surface of KCl-injected sea urchins, were diluted with 1-2 volumes of cold 0.5 M NaCl, instead of being diluted with sea water, as in the Gibbons and Gibbons procedure. Dilution with sea water at this stage can supply sufficient calcium to give symmetrically beating spermatozoa, if the subsequent dilutions are appropriate.

Membrane removal was accomplished with an extraction solution containing 0.15 M KCl, 0.0020 M $MgSO_4$, 0.0020 M Tris buffer, 0.002 M dithiothreitol, 0.0005 M EDTA, 0.04% (vol/vol) Triton X-100, and variable amounts of added $CaCl_2$. Sperm motility was observed in reactivation solutions containing 0.15 M KCl, 0.020 M Tris buffer, 0.002 M dithiothreitol, 2% (wt/vol) polyethylene glycol (carbowax), and variable constituents (EDTA, EGTA, ATP, $MgSO_4$, $CaCl_2$) as indicated in Table I. Equilibrium constants for binding of calcium and magnesium ions by ATP, EDTA and EGTA were obtained from published work (14) and adjusted for temperature and ionic strength when possible. Concentrations of the variable constituents were calculated to

TABLE I

Control of free calcium concentration in the reactivation solutions.

Desired concentration of free Ca^{2+} ions:	Total concentrations added to solution: ATP	$MgSO_4$	$CaCl_2$
Solutions containing 0.0020 M EDTA:			
10^{-3} M	.0020 M	.00151 M	.0038 M
10^{-4}	.00128	.00159	.00209
10^{-5}	.00121	.00211	.0014
10^{-6}	.00120	.00313	.00037*
10^{-7}	.0012	.00345	.00004*
Solutions containing 0.0020 M EGTA:			
10^{-7}	.0012	.0015	.00194
10^{-8}	.0012	.0015	.00151
10^{-9}	.0012	.0015	.00047*

*In making up these solutions, allowance was made for the addition of calcium from the extraction solution.

maintain the $MgATP^{2-}$ concentration at 0.0010 M and the Mg^{2+} concentration at 0.00050 M in each solution, and the desired concentrations of free Ca^{2+} ions. Most of the experiments at 10^{-7} M Ca^{2+} were carried out with the EGTA solution; experiments using the EDTA solution gave identical results.

All preparations and observations were carried out in a room maintained at 16°C. Extraction and reactivation solutions were adjusted to pH 8.0 at 16°C. A 10 microliter sample of NaCl-diluted spermatozoa was mixed with 200 microliters of extraction solution, agitated gently, and incubated for 30 seconds. A portion of this suspension was then diluted with at least 100 volumes of reactivation solution.

Our results are shown in Figure 1. The use of turning rate as a measure of the effects of calcium on the flagellar beat pattern leads to a highly skewed distribution of measured values when the preparations contain

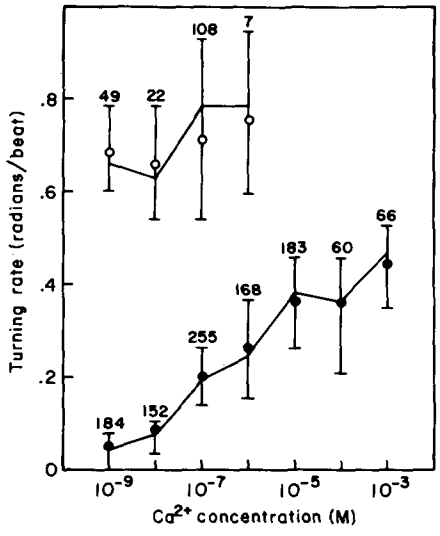

FIGURE 1. Turning rate of spermatozoa as a function of the calcium ion concentration in the reactivation solution. The upper curve (open circles) was obtained with spermatozoa extracted in Triton solutions containing no added calcium. The lower curve (solid circles) was obtained with spermatozoa extracted in Triton solutions containing 0.005 M $CaCl_2$. The interquartile range, median value (connecting lines), mean value (circles), and number of measurements are shown for each distribution.

many spermatozoa with straight swimming paths. The standard deviation is not a valid statistic for these distributions. The presentation of the data in Figure 1 includes points for the mean of each distribution and connecting lines drawn between the median of each distribution. The range shown is the interquartile range, which contains 50% of the measured values. The upper curve in Figure 1 shows the results obtained when the spermatozoa are extracted without adding calcium to the extraction solution. When reactivated at low calcium concentrations, the spermatozoa swim in circular paths and have asymmetrical flagellar beat patterns. As the calcium concentration in the reactivation solution is increased, the asymmetry of beat increases to a point where the path curvature is unmeasurable, and the movement becomes very unstable. Only a few spermatozoa gave measurable paths at 10^{-6} M Ca^{2+}, and these may not be typical of the entire preparation.

The lower curve in Figure 1 shows the results obtained when the extraction solution contains 0.005 M $CaCl_2$. When reactivated at 10^{-9} M Ca^{2+}, about 30% of the spermatozoa swim in paths which have no measurable curvature in 10-20 beats, and the remainder swim in large circles. As the calcium concentration in the reactivation solution is increased, the curvature of the sperm paths increases, with the sharpest change appearing to be in the range of 10^{-7} to 10^{-6} M Ca^{2+}.

Variable results were obtained with intermediate concentrations of calcium in the extraction solutions. With 0.001 M $CaCl_2$ in the extraction solutions, some experiments gave results which fell on a curve clearly intermediate between the two curves in Figure 1. However, in other experiments, results obtained from spermatozoa extracted with 0.001 M $CaCl_2$ were indistinguishable from the results shown for spermatozoa extracted with 0.005 M $CaCl_2$ solutions. There may be other unidentified factors which influence the effect of calcium concentration in the reactivation solution on the symmetry of flagellar beating.

A calcium-activated ATPase, in addition to the Mg-activated dynein ATPase, has been found in Chlamydomonas flagella (15). The direction of ciliary beat in Paramecium has been shown to be regulated by calcium ion concentration, with a region of maximum sensitivity at about 10^{-6} M Ca^{2+} (13). Calcium ions appear to be the mediator of the ciliary responses which are responsible for tactic behavior in Paramecium (7) and have been implicated as a mediator of tactic flagellar responses in Polytoma (1) and in bracken spermatozoids (3). Spermatozoa from a hydroid, Tubularia, develop asymmetrical flagellar beat patterns during chemotactic turning responses (12), and these asymmetrical beat patterns resemble those found in the present experiments with reactivated sea urchin spermatozoa, and with live sea urchin spermatozoa exposed to KCl concentrations greater than normally encountered in sea water (2). However, sea urchin spermatozoa have no known tactic behavior, so a functional significance for calcium regulation of flagellar

beat in these spermatozoa cannot be suggested. The modification of sensitivity to calcium ion concentration by previous exposure to calcium also lacks either a functional or a mechanistic explanation at the present time.

The most immediate application of these results is an improved procedure for working with ATP-reactivated flagellar movements, so that preparations in which spermatozoa are swimming with nearly symmetrical flagellar beat patterns can be used in experimental investigations of the mechanism of flagellar beating.

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