The Elevation of Cyclic AMP Concentrations in Flagella-less Sea Urchin Sperm Heads*  

(Received for publication, August 8, 1980, and in revised form, September 17, 1980) 

David L. Garbers
From the Howard Hughes Medical Institute and the Departments of Pharmacology and Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Cyclic AMP concentrations were elevated and acrosome reactions were induced in intact sea urchin spermatozoa by Nigericin, A23187, and pH 9.0 seawater. To determine whether or not the metabolism of cyclic AMP was being altered in sperm heads, the heads were mechanically separated from the flagella, and the flagella-less heads were then isolated by differential centrifugation. The isolated heads contained 1 to 2 nmol of ATP and 1 to 2 pmol of cyclic AMP/mg wet weight and retained these concentrations for several hours if stored at 0°C. The flagella-less heads also retained the mitochondria of the midpiece area. The heads retained their functional status and could be stimulated to undergo acrosome reactions (filament extension) in response to Nigericin, A23187, or pH 9.0 seawater. Furthermore, the isolated heads could activate sea urchin eggs after induction of an acrosome reaction by Nigericin or pH 9.0 seawater. The isolated heads contained appreciable adenylate cyclase, cyclic AMP phosphodiesterase, cyclic GMP phosphodiesterase, guanylate cyclase, cyclic AMP-dependent protein kinase, and calmodulin. Nigericin, pH 9.0 seawater, and A23187 caused not only the induction of an acrosome reaction but also elevations of cyclic AMP in the isolated heads, and extracellular Ca** was an absolute requirement for both responses. At 16°C, Nigericin caused elevations of cyclic AMP within 5 s, but maximal elevations were not observed until 1 min; it induced a maximal percentage of acrosome reactions by 40 s. Incubation of cells at 0°C resulted in a delay of maximal acrosomal reactions until between 10 and 20 min after addition of Nigericin. Under these conditions, maximal elevations of cyclic AMP were observed by 5 min, demonstrating that cyclic AMP elevations precede the complete morphological change associated with an acrosome reaction.

ATP concentrations within the sperm heads declined in response to Nigericin, pH 9.0 seawater, or A23187, and its decrease also required the presence of extracellular Ca**. The decline in ATP concentrations was slightly more rapid in the presence of rotenone, suggestive of some ATP synthetic capabilities of the isolated head preparation. **Ca** uptake was increased by Nigericin, elevated pH, and A23187 but was not appreciably altered by monensin. Monensin also did not cause appreciable elevations of cyclic AMP concentrations, induction of an acrosome reaction, or decreases of ATP concentrations.

Here, we describe for the first time that cyclic AMP concentrations can be increased in flagella-less heads of spermatozoa and show that these changes are associated with an acrosome reaction.

In 1975, factors associated with sea urchin eggs were shown to cause marked elevations of sea urchin sperm cyclic AMP concentrations (1). Subsequent studies showed that one of the factors (FS-1) responsible for the stimulation of cyclic AMP metabolism was of large molecular size, contained as much as 40% fucose by weight, and required extracellular Ca** for its effects on cyclic AMP (2-5). Physiologically, the purified polymer was shown to induce an acrosome reaction (5). Thus, it appeared possible that cyclic AMP could have a functional role in the development of an acrosome reaction. Such a hypothesis was strengthened by observations that Ca**-transport antagonists such as D-600 could block both FS-1-induced elevations of cyclic AMP and development of an acrosome reaction (5).

It is known that the acrosome reaction of sea urchin spermatozoa can be induced by treatments other than FS-1, including high pH (6), A23187 (7-10), and Nigericin (11). If cyclic AMP metabolism is intimately linked with Ca** transport and the acrosome reaction, its concentrations should be elevated by these treatments. Here, we show that sperm cyclic AMP concentrations, in fact, are increased by pH 9.0 seawater, A23187, or Nigericin but not by monensin, an ionophore that fails to induce a significant number of acrosome reactions over the time periods studied.

Although these data suggest that cyclic AMP is involved with development of an acrosome reaction or with some other physiological change associated with fertilization, the question of whether or not cyclic AMP could actually increase in the sperm head region was not known. Recently, Vacquier (12) reported that sea urchin sperm heads could be separated from flagella after homogenization of cells with a Waring Blender, and that isolated heads retained an intact acrosomal granule. Such heads also retained a relatively intact plasma membrane. Using a modification of his procedure, we prepared sperm heads and separated them from flagella. The heads retained functional status as evidenced by their ability to activate dejellied ovum after treatment to induce an acrosome reaction. The isolated heads responded to Nigericin, A23187, or pH 9.0 seawater with acrosome reactions and also with marked increases of cyclic AMP concentrations. As with intact cells, extracellular Ca** was required for all responses.

EXPERIMENTAL PROCEDURES

Materials—Gametes were obtained from sea urchins purchased from Pacific Bio-Marine, Venice, CA. Tritium-labeled nucleotides were from Amersham or New England Nuclear. All common chemicals were from Sigma Chemical Co. or Fisher Scientific Co. Theophylline was from Merck and Dowex 50 (AG 50W-X8, 100 to 200 mesh)
and neutral alumina (AG-7, 100 to 200 mesh) were from Bio-Rad Laboratories. 3CaCl2 and [γ-32P]ATP were from Amersham. Whatman GF/C glass microfiber filters were from Fisher Scientific Co. or from Beckman. Nigerin, monensin, and A23187 were either gifts from Eli Lilly Co. or purchased from Calbiochem.

Collection of Gametes—Spermatozoa from Strongylocentrotus purpuratus sea urchins were collected and washed as previously described (1). Sperm cells were resuspended to a final concentration of 80 to 120 mg (wet weight/ml) and stored at 0°C until further treatment.

Isolation of Flagella-less Heads—Intact sea urchin sperm heads were obtained essentially by the method of Vacquier (12), except that cells (20 ml) were blended 2 min with an Eberbach Microblender at low speed. Cells also were resuspended as a more concentrated suspension (50 to 75 mg (wet weight/ml)); this resulted in the preparation of greater than 90% intact heads with virtually no attached flagella. After blending, the sperm heads were separated from the detached and broken flagella by centrifugation at 200 × g for 5 min. The pellet, containing the heads, was resuspended to the original volume with seawater and centrifuged again at 200 × g for 5 min. The resultant pellet contained sperm heads (Fig. 1) with no visible contamination by flagella. From approximately 1 g (wet weight) of intact cells, 200 mg (wet weight) of sperm heads are recovered.

Egg Activation—In experiments where activation of the egg (elevation of fertilization membrane) was monitored, collected eggs were first treated with seawater containing 10 mm 2-(N-morpholino)ethanesulfonic acid buffer at pH 5.0 for 30 min. After this treatment, ova were washed by low speed centrifugation (200 × g) twice and resuspended in seawater at pH 7.9. These eggs, deficient or devoid of FS-1, were not activated by sperm heads unless the sperm were first treated to induce an acrosome reaction.

Cyclic Nucleotide Concentrations—The isolated heads or intact cells (50 μl from a suspension containing 30 to 100 mg (wet weight/ml) were added to incubation mixtures containing seawater and various quantities of theophylline, Nigerin, monensin, or other agents, in a final volume of 0.5 ml. Incubations, at 0 or 16°C for various periods of time were stopped by the addition of 1.0 ml of 0.5 n perchloric acid, containing tracer amounts of cyclic [3H]AMP for monitoring cyclic nucleotide recoveries. Zero time cyclic nucleotide concentrations were determined by the addition of spermatozoa directly to seawater containing perchloric acid. The acid-extracted cells were centrifuged, and the resultant supernatant fluid was applied to Dowex 50 columns (0.7 × 15.0 cm) as described by Schultz et al. (13). Cyclic AMP and cyclic GMP were subsequently assayed by the method of Steiner et al. (14) with modifications as described by Harper and Brooker (15).

Enzyme Assays—Adenylate cyclase activity was estimated by the method of Watkins et al. (16), phosphodiesterase activity was estimated by the method of Wells and Garbers (17), cyclic AMP-dependent protein kinase activity was estimated by the method of Garbers et al. (4), and guanylate cyclase activity was estimated by the method of Garbers (18). Product formation was measured as a function of time for all assays.

Calmodulin—The amount of calmodulin present was estimated by its ability to activate a calmodulin-deficient phosphodiesterase from coronary arteries. Purified calmodulin from spermatozoa (19) was used as a standard. Intact cells or isolated sperm heads were placed in a boiling water bath for 10 min to inactivate endogenous phosphodiesterase as well as to release calmodulin. After centrifugation, the supernatant fluids were then tested for ability to activate phosphodiesterase relative to purified calmodulin.

Acrosome Reaction—The percentage of acrosome reactions was determined by estimating filament extensions as described previously (5).

Protein—Protein was estimated by the method of Lowry et al. (20) using bovine serum albumin as a standard.

ATP—ATP concentrations of spermatozoa were estimated using the luciferase assay method as modified by Hammerstedt (21).

Calcium Uptake—Cells or sperm heads were incubated, and the amount of 45Ca2+ taken up was estimated as described previously (5).

RESULTS

Intact Cells—In initial studies, intact sea urchin spermatozoa were incubated with A23187, Nigerin, or pH 9.0 seawater to determine whether or not these agents also caused elevations of cyclic AMP concentrations. All three treatments caused induction of an acrosome reaction and elevations of cyclic AMP (Table I). A23187 resulted in sporadic effects, however, and seldom caused greater than 30% acrosome reactions or more than 8-fold elevations of cyclic AMP. Similar problems with the successful use of this ionophore have been observed by others (11).

These studies indicated not only that a natural molecule (FS-1) responsible for induction of an acrosome reaction could elevate cyclic AMP concentrations, but also that pharmacological treatments capable of induction of an acrosome reaction could elevate sperm cyclic AMP concentrations. To determine whether or not such cyclic AMP elevations could actually occur in the sperm head region, heads that retained functional status were isolated from flagella.

Components of Isolated Sperm Heads—It was first determined whether or not the isolated heads contained the metabolic enzymes associated with cyclic nucleotide metabolism (Table II). Although specific activities for all enzymes were less than that observed in whole cells, the enzyme activities associated with the heads were appreciable. In addition, calmodulin represented about 1.7% of the total head protein.

The isolated sperm heads contained 1 to 2 nmol of ATP/mg wet weight heads and retained these concentrations at 0°C for several hours. At 16°C, the ATP concentrations decreased at a slow rate but decreased more rapidly (~10% greater rate) in the presence of rotenone, suggestive of a low rate of ATP synthesis.

Most of the cytochromes estimated by difference spectra were retained by the heads. This was not unexpected since the mitochondria of sea urchin spermatozoa are localized in the posterior part (midpiece) of the isolated head preparation. These mitochondria are apparently either damaged or essentially nonfunctional, however, since basal respiration rates are about 5% of those estimated for intact cells and, in contrast to intact cells, these low respiration rates are oligomycin-insensitive.

TABLE I

Effect of pH 9.0 seawater, Nigerin, or A23187 on cyclic AMP concentrations and the acrosome reaction of intact sea urchin spermatozoa

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cyclic AMP*</th>
<th>% acrosome reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 s</td>
<td>2 min</td>
</tr>
<tr>
<td>Control</td>
<td>2.1 ± 0.3</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>42 ± 5.2</td>
<td>37 ± 3.1</td>
</tr>
<tr>
<td>Nigercin</td>
<td>28.9 ± 7.3</td>
<td>39 ± 0.5</td>
</tr>
<tr>
<td>A23187</td>
<td>2.3 ± 0.2</td>
<td>8.4 ± 0.6</td>
</tr>
</tbody>
</table>

*Expressed as nanomoles of cyclic AMP/g wet weight cells.

Fig. 1. Schematic drawing of a typical isolated sperm head obtained by the method described under "Experimental Procedures."
Basal cyclic AMP concentrations were 1 to 2 nmol/g wet weight of isolated heads; this compares to concentrations of 1 to 2 nmol of cyclic AMP/g wet weight of intact cells. Cyclic GMP concentrations of isolated heads were generally not detectable (<50 fmol/mg wet weight), despite appreciable guanylate cyclase activity associated with the heads.

Isolated sperm heads, then, not only contain cyclic AMP but also contain the necessary components for the metabolism of cyclic nucleotides.

Effects of pH 9.0 Seawater—Elevated pH not only caused induction of the acrosome reaction in the isolated heads, but also caused large (50- to 100-fold) increases of cyclic AMP concentrations (Fig. 2A). Maximal elevations of cyclic AMP were observed at 1 min after addition of sperm heads to the pH 9.0 seawater. However, if Ca\(^{2+}\) were removed from the seawater, the elevated pH had little, if any, effect on either the percentage of acrosome reactions or cyclic AMP elevations (Fig. 2A).

ATP concentrations declined in response to the elevated pH but, as with cyclic AMP elevations, no significant decrease in ATP was observed unless extracellular Ca\(^{2+}\) was present (Fig. 2B). In contrast to the effects of pH 9.0 seawater on cyclic AMP concentrations, where concentrations increased until 1 min then declined, ATP concentrations continued to decline as a function of time.

**Table II**

Specific activity of various enzymes related to cyclic nucleotide metabolism and of calmodulin of sea urchin sperm cells or of isolated heads

Sperm heads were prepared and enzyme activities or calmodulin were estimated as described under “Experimental Procedures.” With guanylate cyclase, the enzyme activity was estimated in the presence of seawater, the elevated pH but, as with cyclic AMP elevations, no significant decrease in ATP was observed unless extracellular Ca\(^{2+}\) was present (Fig. 2B). In contrast to the effects of pH 9.0 seawater on cyclic AMP concentrations, where concentrations increased until 1 min then declined, ATP concentrations continued to decline as a function of time.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Specific activity</th>
<th>Intact cells</th>
<th>Isolated heads</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol product/min/mg protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guanylate cyclase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Triton X-100</td>
<td>5.7 ± 0.58 (8)</td>
<td>1.9 ± 0.32 (8)</td>
<td></td>
</tr>
<tr>
<td>+ Triton X-100</td>
<td>36.6 ± 3.8 (8)</td>
<td>15.5 ± 4.6 (8)</td>
<td></td>
</tr>
<tr>
<td>Adenylate cyclase</td>
<td>4.9 ± 0.17 (8)</td>
<td>1.7 ± 0.31 (8)</td>
<td></td>
</tr>
<tr>
<td>Protein kinase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Cyclic AMP</td>
<td>0.08 ± 0.01 (4)</td>
<td>0.04 ± 0.01 (4)</td>
<td></td>
</tr>
<tr>
<td>+ Cyclic AMP</td>
<td>0.46 ± 0.05 (4)</td>
<td>0.16 ± 0.01 (4)</td>
<td></td>
</tr>
<tr>
<td>Cyclic AMP phosphodiesterase</td>
<td>0.75 (2)</td>
<td>0.25 (2)</td>
<td></td>
</tr>
<tr>
<td>Cyclic GMP phosphodiesterase</td>
<td>3.3 (2)</td>
<td>0.87 ± 0.11 (4)</td>
<td></td>
</tr>
<tr>
<td>Calmodulin*</td>
<td>2.35 (2)</td>
<td>1.74 (2)</td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as micrograms of calmodulin/mg of protein.

D-900, a calcium-transport antagonist, partially blocked acrosome reactions, cyclic AMP elevations, and ATP reductions in response to pH 9.0 seawater (Table III). However, these concentrations of D-600 would completely block acrosome reactions in response to FS-1 at pH 8.0 (5), suggesting that the drug is much less effective at pH 9.0.

The uptake of \(^{45}\)Ca\(^{2+}\), normally observed in intact cells in response to FS-1 (5) or Nigericin (11), was also observed in response to elevated pH in the isolated sperm heads (Table III). The apparent uptake was not due to \(^{45}\)Ca\(^{2+}\) precipitation at pH 9.0.

Effects of Nigericin—As with elevated pH, Nigericin caused induction of the acrosome reaction and also caused marked elevations of cyclic AMP in the isolated heads (Fig. 3A). Again, extracellular Ca\(^{2+}\) was required for the responses to Nigericin. ATP concentrations remained relatively stable in the absence of Ca\(^{2+}\) but declined rapidly in the presence of extracellular Ca\(^{2+}\) in the presence of Nigericin (Fig. 3B). Nigericin also caused significant \(^{45}\)Ca\(^{2+}\) uptake by isolated heads (Table III).

Since acrosome reactions are delayed until approximately 40 s after addition of Nigericin at 16°C, experiments were designed to estimate acrosome reactions and cyclic AMP concentrations at short time points to determine whether cyclic AMP was elevated prior to filament extension. At 16°C, cyclic AMP concentrations were elevated approximately 10-fold by 10 s and were maximally elevated by 1 min (Fig. 4B). Acrosome reactions as evidenced by filament extension were initiated at 10 s and reached maximal percentages at 40 s. Because of the difficulty in determining whether or not the cyclic AMP elevations actually preceded the acrosome reaction, the incubation temperature was reduced to 0°C. Under these conditions, maximal acrosome reactions were delayed until 10 to 20 min after Nigericin addition (Fig. 3A). Cyclic AMP elevations, in contrast, were significantly elevated within 1 to 2 min and reached maximal concentrations by 5 min (Fig. 3A). Thus, cyclic AMP elevations appear to precede filament extension.

Effects of A23187—As reported by Schackmann et al. (11), the effects of this ionophore were variable. Even in Mg\(^{2+}\)-free seawater, the ionophore rarely induced more than 30% acrosome reactions. At 30 s after addition of 33 μM A23187 to the sperm heads, the mean cyclic AMP concentration had increased from 0.72 ± 0.04 (n = 4) to 7.40 ± 0.04 (n = 4) nmol/g wet weight cells. The concentrations remained elevated and were 6.9 ± 0.2 (n = 4) at 1 min and 6.9 ± 0.3 (n = 4) nmol/g wet weight cells at 2 min after addition of the ionophore. The mean percentages of acrosome reactions were 1%, 1%, 12%, and 25% at 0, 30 s, 1 min, and 2 min after addition of A23187.

---

**Fig. 2. Effect of pH 9.0 seawater on cyclic AMP and ATP concentrations and on the acrosome reaction of isolated sea urchin sperm heads.**

Isolated sperm heads prepared in Ca\(^{2+}\)-free seawater at pH 7.9 were added to pH 9.0 seawater at zero time, and reactions were stopped at the times indicated by the addition of 1 ml of 0.5 N perchloric acid or of 1 ml of 2% glutaraldehyde. In instances where Ca\(^{2+}\) was present, it was added to a final concentration of 9.6 mm. The numbers in parentheses represent the percentages of acrosome reactions for the respective treatments, and the values given are the means ± S.E. (n = 4).
Cyclic AMP in Sperm Heads

623

Table III
Effect of Nigericin, monensin, and pH 9.0 seawater on cyclic AMP and ATP concentrations, on 45Ca2+ uptake, and on percentage of acrosome reactions of isolated sea urchin sperm heads

Isolated heads were incubated in the presence or absence of D-600 (200 µM) or solvent (control), pH 9.0 seawater, Nigericin (30 µM), or monensin (30 µM). Cyclic AMP and ATP concentrations were determined at 1 and 5 min, respectively. The values given represent the means ± S.E., and the values in parentheses are the numbers of determinations.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>D-600, 200 µM</th>
<th>Cyclic AMP⁴</th>
<th>ATP³</th>
<th>Acrosome reactions</th>
<th>⁴Ca2+ uptake⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>2.1 ± 0.05 (12)</td>
<td>1.07 ± 0.05 (9)</td>
<td>3.5 ± 1 (6)</td>
<td>0.10 ± 0.07 (4)</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>+</td>
<td>2.0 ± 0.10 (8)</td>
<td>1.03 ± 0.04 (12)</td>
<td>5.0 ± 2 (4)</td>
<td>0.05 ± 0.002 (4)</td>
</tr>
<tr>
<td>Nigericin</td>
<td>-</td>
<td>182 ± 6 (8)</td>
<td>0.58 ± 0.02 (12)</td>
<td>58 ± 4 (7)</td>
<td>1.02 ± 0.14 (4)</td>
</tr>
<tr>
<td>Monensin</td>
<td>-</td>
<td>35 ± 2 (8)</td>
<td>0.88 ± 0.02 (8)</td>
<td>18 ± 3 (6)</td>
<td>0.50 ± 0.08 (4)</td>
</tr>
</tbody>
</table>

⁴ Expressed as nanomoles of cyclic AMP/g wet weight.
³ Expressed as micromoles of ATP/g wet weight.
² Expressed as nanomoles of ⁴Ca accumulated/mg wet weight during a 5-min incubation.

Fig. 3. Effect of Nigericin on cyclic AMP and ATP concentrations and on acrosome reactions of isolated sea urchin sperm heads. Isolated sperm heads were added to seawater containing 30 µM Nigericin or an equivalent amount of added solvent. The incubation conditions were otherwise as described in the legend to Fig. 1. The numbers in parentheses equal the percentages of acrosome reactions for the respective treatments, and the values given are the means ± S.E. (n = 4).

Fig. 4. Time course of cyclic AMP elevations and of acrosome reactions of isolated sea urchin sperm heads in response to Nigericin at 16° or 0°C. Isolated heads were incubated in the presence of 30 µM Nigericin at either 0° or 16°C and, at the time points indicated, heads were treated with 1 ml of 0.5 N perchloric acid or with 1 ml of 2% glutaraldehyde and cyclic AMP concentrations, or acrosome reactions were estimated. △ and ○, cyclic AMP concentrations ± S.E. (n = 8); □ and ●, percentage of acrosome reactions ± S.E. (n = 8).

After 5 min of incubation, the mean percentage of acrosome reactions had stabilized at 31 and the mean concentration of cyclic AMP had decreased to 5.4 ± 0.8 (n = 4) nmol/g wet weight cells. These effects of A23187 were subsequently shown to also completely depend on extracellular Ca2+.

Effects of Monensin—Monensin, an ionophore that catalyzes electrically neutral Na+-H+ exchange, in contrast to Nigericin did not induce a significant number of acrosome reactions over the 5-min time period studied (Fig. 5). Monensin also had little, if any, effect on sperm cyclic AMP or ATP concentrations in the presence or absence of extracellular Ca2+ (Fig. 5).

Egg Activation—In the final experiments, the isolated sperm heads were added to dejellied eggs to determine whether or not induction of an acrosome reaction would result in heads capable of activating an egg. Heads treated with both Nigericin or elevated pH seawater were tested. When sperm
heads not treated to induce an acrosome reaction were added to dejellied eggs (approximately 1000 sperm heads added/egg), less than 0.5% of the eggs showed elevation of a fertilization membrane, but when sperm cells were treated with Nigericin or pH 9.0 seawater, 50 to 80% of all eggs showed elevation of the fertilization membrane if sperm cells and eggs were mixed together within 40 s after completion of the acrosome reaction (not shown). Thus, the acrosome reactions induced by Nigericin or pH 9.0 seawater represent functional reactions.

If eggs with jelly were used, however, no egg activation occurred and sperm heads failed to penetrate the jelly layer, suggesting that motility is required for penetration of the matrix (jelly) surrounding the egg. Motility, then, is apparently not required for actual sperm-egg fusion.

**DISCUSSION**

These studies show that flagella-less sperm heads respond to pH 9.0 seawater, Nigericin, or A23187 with filament extensions, marked elevations of cyclic AMP, and reductions of ATP, and that all responses depend on extracellular Ca++. The induction of an acrosome reaction by these treatments results in sperm heads capable of activating dejellied eggs, suggesting that the acrosome reactions induced by these agents are similar to, or the same as, reactions induced naturally by egg jelly. The elevations of cyclic AMP and the reductions of ATP in isolated sperm heads represent the first descriptions of distinct biochemical changes in heads which are correlated with the development of an acrosome reaction. As with intact cells, Ca++ appears to function as the primary initiator of the events associated with an acrosome reaction.

At 16°C, the acrosome reaction and the elevation of cyclic AMP occur rapidly in response to Nigericin, but at 0°C, the effects of Nigericin are considerably delayed. The increased period of time required for an acrosomal response at 0°C resulted in data showing that cyclic AMP elevations can clearly precede the extension of the acrosomal filament. The complete morphological change termed the acrosome reaction no doubt involves the coordination of many enzymatic events. That cyclic AMP concentrations increase prior to the complete morphological change suggests that the nucleotide could have a functional role in the process. It is obvious from these studies that the enzymes associated with cyclic nucleotide metabolism are present in the head region, and that appreciable quantities of calmodulin are also present. Thus, some or all of the Ca++ effects in the sperm head region could be mediated by a Ca++-calmodulin complex. The data do not support the contention of one report (22), however, that calmodulin is located almost exclusively in the acrosomal area. If this were the case, the isolated heads should have shown enrichment of calmodulin compared to intact cells. Other recent work based on immunofluorescent techniques has localized calmodulin in at least four different regions of rabbit and guinea pig spermatozoa (23); our results would also tend to support a more diffuse distribution of calmodulin. Although the function of the cyclic AMP in the head region remains to be determined, activation of the cyclic AMP-dependent protein kinase and resultant protein phosphorylation probably represent the primary response to its elevation. In intact cells, FS-1 is known to activate the cyclic AMP-dependent protein kinase, and maximal enzyme activations are observed in response to increases of cellular cyclic AMP concentrations of 5- to 6-fold (4). Thus, the large increases of cyclic AMP observed in response to pH 9.0 seawater, Nigericin, or FS-1 probably reflect, in part, pharmacological increases.

It also has been suggested that cyclic AMP may have some role in the development of capacitated and/or acrosome-reacted mammalian spermatozoa (24-28), although the evidence is only corollary at this time. Recently, others have suggested that cyclic GMP has a functional role in development of an acrosome reaction (29). Although this may be the case in mammalian spermatozoa, cyclic GMP may not be involved in the acrosome reaction of sea urchin spermatozoa, since FS-1 induces an acrosome reaction but fails to significantly elevate cyclic GMP concentrations (5).

**Acknowledgments**— I express appreciation to D. Janette Tubb for excellent technical assistance on this project, Dr. Jack N. Wells for calmodulin-deficient phosphodiesterase, and Dr. G. S. Kopf for conducting some of the preliminary experiments.

**REFERENCES**

27. Fraser, L. R. (1979) J. Reprod. Fertil. 67, 377-384