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Regulation of Guinea Pig Sperm Adenylate Cyclase by Calcium[1]

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ABSTRACT

The effect of various metal ions on guinea pig sperm adenylate cyclase activity was determined. In the presence of 4.5 mM free metal, relative enzyme activity with Mn^{2+}, Ca^{2+}, Mg^{2+}, Co^{2+}, Zn^{2+}, and Ba^{2+} was 1.00, 0.16, 0.10, 0.10, 0.05 and 0.02, respectively. Added Ca^{2+}, specifically, appeared to activate the enzyme in the presence of Mn^{2+} or Mg^{2+}. The guinea pig sperm adenylate cyclase was stimulated ∿4-fold by low concentrations (μM) of free Ca^{2+} in the presence of Mg^{2+} (5 mM). This Ca^{2+}-dependent increase in adenylate cyclase activity was inhibited by trifluoperazine (0.3–0.5 mM), a known inhibitor of calmodulin. Basal adenylate cyclase activity measured in the presence of Mg^{2+} (5 mM) and in the absence of Ca^{2+} was not affected by the addition of trifluoperazine (0.5 mM). Treatment of the sperm homogenate with ethylene-glycol-bis (β-aminoethyl ether) N,N′-tetra-acetic acid (EGTA) under a variety of conditions failed to completely remove the Ca^{2+}-sensitivity of the particulate adenylate cyclase; such treatment also failed to remove the membrane associated calmodulin. After detergent solubilization, the sperm Mg^{2+}-dependent adenylate cyclase activity was less than 0.5% of the Mn^{2+}-dependent activity and was not stimulated by added Ca^{2+}. These results suggest that a component of the guinea pig sperm adenylate cyclase complex is regulated by Ca^{2+}. Whether the Ca^{2+}-sensitive component is calmodulin remains unclear.

INTRODUCTION

Altered Ca^{2+} permeability may represent one of the primary signals for induction of the acrosome reaction in spermatozoa (Yanagimachi and Usui, 1974; Yanagimachi, 1975; Talbot et al., 1976; Singh et al., 1978). Added Ca^{2+} may also cause an elevation of cyclic AMP concentrations in both hamster (Morton et al., 1974) and guinea pig (Hyne and Garbers, 1979) spermatozoa. Although the biochemical mechanism of the Ca^{2+}-induced elevation of cyclic AMP concentrations was not established in the previous studies (Morton et al., 1974; Hyne and Garbers, 1979), the phosphodiesterase inhibitor, 1-methy-3-isobutylxanthine, acted synergistically with Ca^{2+} to elevate cyclic AMP concentrations in guinea pig spermatozoa (Hyne and Garbers, 1979), suggesting that the addition of Ca^{2+} results in an activation of adenylate cyclase.

In some tissues, enzymes that control cyclic nucleotide metabolism are known to be regulated by a Ca^{2+}-dependent protein referred to as calmodulin or Ca^{2+}-dependent regulatory protein (Cheung, 1970; Kakiuchi and Yamazaki, 1970). Spermatozoa were shown some years ago to contain a Ca^{2+} binding protein (Brooks and Siegel, 1973) and this was subsequently identified as calmodulin based on its ability to activate cyclic nucleotide phosphodiesterase from tissues other than spermatozoa (Wells and Garbers, 1976; Jones et al., 1978). The sperm calmodulin, however, did not appear to activate cyclic nucleotide phosphodiesterases obtained from spermatozoa (Wells and Garbers, 1976). These calmodulins are thought to be responsible for a number of Ca^{2+}-dependent events in addition to their stimulation of cyclic nucleotide phosphodiesterase, including the activation of brain adenylate cyclase (Brostrom et al., 1975), troponin-C-like activity with a reconstituted Ca^{2+}-sensitive actomyosin ATPase (Amphlett et al., 1976; Dedman et al., 1977), activation of erythrocyte membrane (Ca^{2+}, Mg^{2+}) ATPase (Jarrett and Penniston, 1977; Gopinath and Vincenzi, 1977), stimulation of erythrocyte membrane Ca^{2+} transport (Hinds et al., 1978), stimulation of Ca^{2+} transport in cardiac microsomal preparations (Katz and Remtulla,

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1978), activation of smooth muscle myosin light chain kinase (Dabrowska et al., 1977, 1978), skeletal muscle myosin light chain kinase (Yagi et al., 1978) and brain protein kinase (Schulman and Greengard, 1978), stimulation of glycogen synthase phosphorylation (Srivastava et al., 1979) and probable regulation of the Ca^{2+}-dependent skeletal muscle phosphorylase kinase which contains calmodulin as a subunit (Cohen et al., 1978).

Because of the reported Ca^{2+}-dependent elevation of cyclic AMP concentrations in hamster and guinea pig spermatozoa (Morton et al., 1974; Hyne and Garbers, 1979) we investigated the effects of Ca^{2+} on guinea pig sperm adenylate cyclase activity, with emphasis on the potential regulation of the enzyme by calmodulin.

MATERIALS AND METHODS

Materials

Materials were purchased from the following suppliers: [2-^{3}H]-ATP (15 Ci/mmol), Amersham; unlabeled nucleotides, Sigma or United States Biochem. Corp.; metal chlorides, Fisher; creatine phosphate and creatine kinase, Boehringer Mannheim; Dowex-50 (AG 50W-X8, 100–200 mesh) and Neutral Alumina (AG 7, 100–200 mesh), Bio-Rad; Aqueous Counting Scintillant (ACS), Amersham; Lubrol WX, Sigma; Triton X-100, Research Products International; Phenyl-Sepharose, DEAE-Sephacel and Sephadex G-100, Pharmacia; trifluoperazine HCl, Smith, Kline and French Laboratories; sea urchins (*Strongylocentrotus purpuratus*), Pacific Bio-Marine, Venice, CA.

Enzyme Preparation

Guinea pig spermatozoa from the distal portion of the caudal epididymis were isolated after flushing the lumen of the epididymis with 0.9% NaCl in a syringe fitted with a 22 gauge needle as described previously (Hyne and Garbers, 1979). The spermatozoa were washed once by centrifugation (700 × g for 15 min) and resuspended in a hypotonic buffer containing 10 mM NaCl, 10 mM KCl, 0.1 mM dithiothreitol, 0.005 mM EDTA and 2 mM Tris, pH 7.9. The final sperm concentration in the hypotonic buffer was 0.5 × 10^8 spermatozoa/ml. The cells were homogenized with 20 strokes in a tight fitting glass Douce homogenizer on ice and then frozen in 1 ml aliquots at −70°C. The enzyme was stable at −70°C for at least 2 months; enzyme not used after thawing was discarded.

Homogenates of guinea pig spermatozoa were separated into particulate and supernatant fractions by centrifugation at 40,000 × g for 60 min.

Detergent Dispersed Adenylate Cyclase

Guinea pig sperm homogenates were suspended in hypotonic buffer as described above, followed by the addition of Lubrol WX or Triton X-100 to give a 0.3% or 1.0% final detergent concentration. After the suspension containing detergent was allowed to stand at 0–2°C for 30 min, it was centrifuged at 40,000 × g for 30 min. The supernatant fluid and the pellet (resuspended to the original volume in hypotonic buffer) were then assayed for adenylate cyclase activity.

Assay

The general adenylate cyclase assay mixture contained 30 mM Tris at pH 7.9, 2.2 mM cyclic AMP, 7 mM creatine phosphate, 305 μg/ml creatine kinase, 0.5 mM ATP, 5 mM $MgCl_2$ (or 5 mM $MnCl_2$), homogenate from 2.5 × 10^6 spermatozoa and 11–34 × 10^6 dpm of [^{3}H]-ATP in a final volume of 0.25 ml. Enzyme reactions were linear as a function of added protein at 30°C under these conditions and were linear with time for at least 15 min. Concentrations of other additives are specified in the legends to the figures. Reactions were terminated after 10 min of incubation by the addition of 2 ml 0.5 M perchloric acid and the cyclic AMP was then purified on acid alumina and neutral Dowex-50 (H^+-form) columns (Jacobs et al., 1976). Of the 6 ml collected from the Dowex-50 column, 1 ml was used for the determination of unlabeled cyclic AMP (absorbance at 259 nm) and 5 ml was added to 10 ml of ACS (Amersham) and counted for radioactivity. Cyclic nucleotide recoveries, after purification, were 50–65%.

Preparation of Calmodulin

The calmodulin from sea urchin spermatozoa was purified to homogeneity based on sodium dodecyl-SO_4 polyacrylamide gel electrophoresis. Purified samples representing up to 75 μg of added protein, migrated as a single protein staining band on 12% gels. The purified material activated calmodulin deficient phosphodiesterase from coronary arteries in a calcium-dependent manner, had a molecular weight of ∿17,800 and had an amino acid composition similar to calmodulin purified from other tissues (Teo et al., 1973; Stevens et al., 1976; Watterson et al., 1976). Basically, the calmodulin purification procedure was as follows: sea urchin spermatozoa (1.82 kg, wet weight) were suspended and homogenized in 4000 ml of a solution containing 25 mM triethanolamine and 5 mM dithiothreitol. The resulting suspension of broken cells was centrifuged at 11,000 × g for 12 h at 2°C and the supernatant fluid was saved. The supernatant fluid was adjusted with solid $(NH_4)_2SO_4$ to 50% saturation (4°C) and centrifuged at 19,200 × g for 12 h. The supernatant fluids containing the calmodulin were than adjusted to pH 4.2 with HCl and centrifuged at 19,200 × g for 2 h. The resulting pellets were dissolved in 750 ml 25 mM triethanolamine at pH 7.6 and heated at 85°C for 10 min. The precipitate was removed by centrifugation and the supernatant fluid added to a DEAE-Sephacel column (5 × 16 cm) equilibrated with the 25 mM triethanolamine buffer at pH 7.6. Calmodulin was eluted from the column with a linear $(NH_4)_2SO_4$ gradient. Calmodulin was monitored both by its ability to activate calmodulin deficient phosphodiesterase and by its migration pattern on Na-dodecyl-SO_4 polyacrylamide gels. The fractions

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containing calmodulin were pooled, dialyzed extensively against distilled H_2O and lyophilized. The lyophilized material was dissolved in a solution containing 25 mM triethanolamine at pH 7.6, 1 mM EGTA and 0.1 M NaCl and applied to a Sephadex G-100 column (2.6 X 34 cm). The fractions containing calmodulin activity were pooled, dialyzed extensively against distilled H_2O and lyophilized. The lyophilized calmodulin at this step appeared homogeneous and was used in the adenylate cyclase experiments.

Calmodulin Deficient Phosphodiesterase

The calmodulin deficient phosphodiesterase was prepared from coronary arteries as described by Wells et al. (1975). This form of the phosphodiesterase is activated 3–4-fold by the Ca^{2+}-calmodulin complex.

RESULTS

Effect of Metal Ions on Adenylate Cyclase Activity of Sperm Homogenate

Maximal activity of the guinea pig sperm adenylate cyclase was observed in the presence of Mn^{2+} under conditions where $[Mn^{2+}] > [ATP]$. Enzyme activity was low or not detectable in the presence of 5 mM Ba^{2+} or Zn^{2+} whereas Mg^{2+}, Ca^{2+} or Co^{2+} supported rates of 10–16% relative to the activities observed with Mn^{2+} (Table 1). In the presence of Mn^{2+}, Mg^{2+} or Co^{2+}, the addition of Ca^{2+} resulted in apparent increases in adenylate cyclase activity. Co^{2+} and Mg^{2+}, however, did not augment the enzyme activity observed in the presence of Mn^{2+}. The effect of 5 mM Ca^{2+} in combination with 5 mM Mn^{2+} or Mg^{2+} on adenylate cyclase activity was greater than the sum of the individual effects of the metals (Table 1) and was also greater than the effects observed with 10 mM Mn^{2+} or 10 mM Mg^{2+} (data not shown).

Effect of Ca^{2+} Concentration on Adenylate Cyclase Activity of Sperm Homogenate

Homogenates of guinea pig spermatozoa were assayed for adenylate cyclase activity in incubation mixtures containing 200 μM ethyleneglycol-bis (β-aminoethyl ether) N,N'-tetra-acetic acid (EGTA), 5 mM Mg^{2+} and various concentrations of added Ca^{2+} (Fig. 1). As the Ca^{2+} concentration approached and exceeded the concentration of EGTA in the incubation medium, a stimulation of adenylate cyclase activity was detected. An $\sim$4-fold stimulation of adenylate cyclase activity was observed at a total Ca^{2+} concentration of 240 μM; when the total Ca^{2+} concentration was increased to 500 μM or greater, adenylate cyclase activity appeared to be stimulated even further (Fig. 1).

Effect of Trifluoperazine on the Ca^{2+}-dependent Adenylate Cyclase Activity of the Sperm Homogenate

The stimulation of the adenylate cyclase by micromolar concentrations of Ca^{2+} suggested the possible involvement of calmodulin as an intermediary of the Ca^{2+} effect. Adenylate cyclase activity estimated in the presence of 5 mM Mg^{2+} and 200 μM EGTA was classified as Ca^{2+}-independent activity, while the difference between this activity and the activity estimated in the presence of Mg^{2+}, EGTA and Ca^{2+} was defined as Ca^{2+}-dependent adenylate cyclase activity. To determine whether the Ca^{2+}-

TABLE 1. Adenylate cyclase activity of guinea pig sperm homogenates in the presence of various divalent cations.

Divalent cation	Adenylate cyclase activity[a] (pmol cyclic AMP formed/min/10^8 spermatozoa
Mn^{2+}	463 ± 13
Mg^{2+}	44 ± 5
Ca^{2+}	74 ± 16
Ba^{2+}	7 ± 5
Co^{2+}	45 ± 12
Zn^{2+}	22 ± 16
Mn^{2+} + Ca^{2+}	647 ± 47
Mg^{2+} + Ca^{2+}	224 ± 31

[a]Adenylate cyclase reaction mixtures contained the divalent cations at a total concentration of 5.0 mM in the presence of 0.5 mM ATP and were otherwise as described in Materials and Methods. Values are the mean ± SEM for 3 animals.

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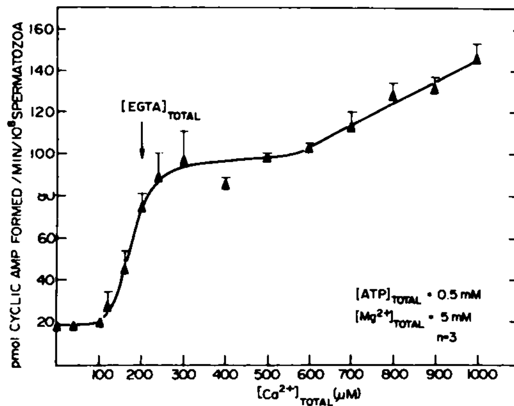

FIG. 1. The effect of various concentrations of Ca^{2+} on guinea pig sperm adenylate cyclase in the presence of 5 mM Mg^{2+}. Reaction mixtures contained 0.5 mM ATP, 5.0 mM total Mg^{2+}, 200 μM EGTA and were as described in Materials and Methods except that the total Ca^{2+} concentration was varied as shown. Values are the mean ± SEM for 3 animals.

dependent adenylate cyclase activity was regulated by calmodulin, the homogenates of guinea pig spermatozoa were assayed for adenylate cyclase activity in the presence of added Ca^{2+} and various concentrations of trifluoperazine. Phenothiazines such as trifluoperazine inhibit the calmodulin stimulations of cyclic nucleotide phosphodiesterases (Levin and Weiss, 1976, 1977) and of adenylate cyclase (Brostrom et al., 1977). Approximately

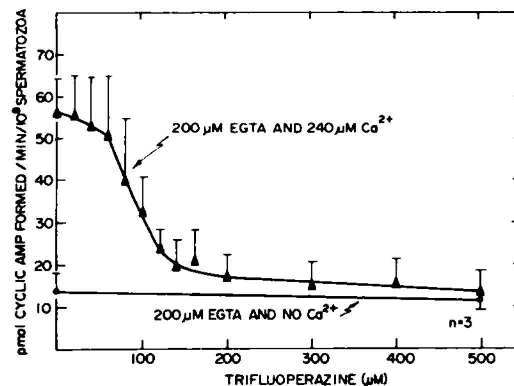

FIG. 2. Inhibition of the Ca^{2+}-dependent adenylate cyclase activity of guinea pig spermatozoa by trifluoperazine. Adenylate cyclase reaction mixtures contained 0.5 mM ATP, 5.0 mM total Mg^{2+}, 200 μM EGTA and were as described in Materials and Methods except that 240 μM Ca^{2+} (final concentration) was added to some of the reactions and various concentrations of trifluoperazine were added as indicated. Values are the mean ± SEM for 3 animals.

100 μM trifluoperazine inhibited the Ca^{2+}-dependent adenylate cyclase activity by 50% (Fig. 2). In contrast, the Ca^{2+}-independent adenylate cyclase activity was not significantly affected by trifluoperazine up to concentrations of 500 μM (Fig. 2). Treatment of the guinea pig sperm homogenate with 100 μM trifluoperazine in a medium containing 5 mM Mg^{2+}, 200 μM EGTA and various concentrations of added Ca^{2+} also resulted in an inhibition of adenylate cyclase activity; however, inhibition was not apparent in the absence of free Ca^{2+} (Fig. 3). In the presence of free Ca^{2+}, 100 μM trifluoperazine inhibited ∿50%, independent of the Ca^{2+} concentration (Fig. 3). The addition of 500 μM trifluoperazine to the guinea pig sperm homogenate in an incubation medium containing 4.5 mM of free Ca^{2+} as the only divalent cation also inhibited the adenylate cyclase activity by ∿50% (data not shown).

Regulation of Particulate Adenylate Cyclase by Ca^{2+}

Since mammalian spermatozoa have been reported to contain both a soluble and a particulate form of adenylate cyclase (Herman et al., 1976), spermatozoa were fractionated to determine which form(s) exhibited the Ca^{2+}-dependent behavior.

Homogenates of guinea pig spermatozoa were separated into particulate and supernatant fractions by centrifugation at 40,000 × g for 60 min. Only 5–10% of the enzyme activity was recovered in the supernatant fluid in the presence of Mg^{2+} (Table 2) or Mn^{2+} (data not shown). The Ca^{2+}-sensitive adenylate cyclase activity appeared to be restricted to the particulate fraction (Table 2), although activities were so low in the supernatant fluid that slight increases in activity due to Ca^{2+} would have been difficult to estimate.

When the sperm homogenate was centrifuged in the presence of 200 μM EGTA and then resuspended in hypotonic buffer, the addition of 240 μM Ca^{2+} in the presence of 5 mM Mg^{2+} and 200 μM EGTA resulted in a 2–3-fold stimulation of adenylate cyclase activity (data not shown). This compares with a 4-fold stimulation of the enzyme in particles not treated with EGTA. Sonication of the sperm homogenate (Branson Sonifier, Model LS 75, setting 4, 10 sec) prior to centrifugation, or the washing of the particles twice with 0.2 mM EGTA by centrifugation, also failed to remove Ca^{2+}-sensitivity of the particu-

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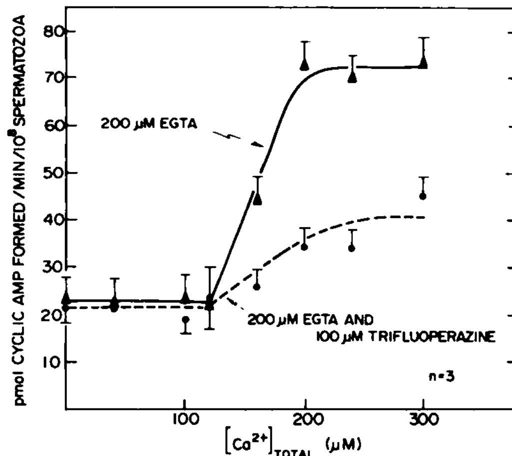

FIG. 3. Effect of trifluoperazine on the adenylate cyclase activity of guinea pig spermatozoa at various concentrations of Ca^{2+}. Reaction mixtures contained 0.5 mM ATP, 5.0 mM total Mg^{2+}, 200 μM EGTA and were as described in Materials and Methods except that 100 μM trifluoperazine (final concentration) was added to some of the reactions and the total Ca^{2+} concentration was varied as shown. Values are the mean ± SEM for 3 animals.

late form of adenylate cyclase. The addition of exogenous, purified sea urchin sperm calmodulin (3.6–10.9 μg/ml) to the EGTA washed particles did not appear to have any effect on enzyme activity in the presence or absence of Ca^{2+}.

To determine whether calmodulin was removed from the particles by the EGTA treatment, particles were washed twice with 200 μM EGTA, heated at 90°C for 15 min and then assayed for calmodulin activity using a calmodulin-deficient phosphodiesterase from coronary arteries (Wells et al., 1975). At least 1000 ng calmodulin appeared to be present in the particles obtained from the equivalent of 2.5×10^6 cells. Thus, EGTA treatment of the particles was ineffective at removing membrane bound calmodulin.

Detergent Dispersed Adenylate Cyclase

Attempts were made to disperse and solubilize the guinea pig sperm adenylate cyclase activity with the nonionic detergents, Lubrol WX and Triton X-100, as has been reported previously for rat brain (Johnson and Sutherland, 1973) and sea urchin spermatozoa (Garbers, 1977). Guinea pig sperm homogenates were prepared as described in Materials and Methods, then Lubrol WX or Triton X-100 was added to give a 0.3% or 1.0% final detergent concentration. The suspension was allowed to stand at 0–2°C for 30 min and then centrifuged at 40,000 × g for 30 min. When the spermatozoa were treated with Lubrol WX, no adenylate cyclase activity was detected in either the supernatant fluid or the extracted particles in the presence of Mn^{2+}. Thus, the enzyme appeared to be inhibited or inactivated by the Lubrol WX. Adenylate cyclase activity was detectable, however, in the supernatant fluid obtained after treatment of the sperm particles with Triton X-100. Approximately 65% of the Mn^{2+}-dependent enzyme activity present in the particles prior to detergent treatment was recovered in the supernatant fluid. After detergent treatment, the sperm Mg^{2+}-dependent adenylate cyclase activity was less than 0.5% of the Mn^{2+}-dependent activity and neither the Mn^{2+} nor the Mg^{2+}-dependent activities were stimulated by added Ca^{2+} (data not shown). The enzyme solution containing detergent, however, contained substantial amounts of calmodulin based on the ability of a heated

TABLE 2. Adenylate cyclase activity of supernatant and particulate fractions of guinea pig spermatozoa in the presence or absence of free Ca^{2+}.

	Adenylate cyclase activity[a] (pmol cyclic AMP formed/min/10^8 spermatozoa		
$[Ca^{2+}]$ total (μM)	Homogenate	Supernatant	Particulate
0	11.3 ± 3.2	<0.3	11.9 ± 6.8
240	41.3 ± 10.1	<0.3	40.7 ± 11.1

[a]Adenylate cyclase reaction mixtures contained 0.5 mM ATP, 5.0 mM total Mg^{2+}, 200 μM EGTA and were as described in Materials and Methods except that 240 μM Ca^{2+} (final concentration) was added to some of the reactions. Values are the mean ± SEM for 3 animals.

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aliquot of the solution to activate the coronary artery phosphodiesterase. Added calmodulin (3.6 μg/ml) also did not activate the detergent dispersed enzyme.

The calmodulin could be removed by chromatography of the detergent dispersed enzyme on a Phenyl-Sepharose column (0.9 x 5 cm) previously equilibrated with a buffer containing 25 mM triethanolamine, pH 7.6, 1 mM dithiothreitol, 0.2 M NaCl and 1 mM EGTA. The adenylate cyclase remained bound to the column under these conditions, whereas all of the detectable calmodulin activity eluted at the front of the column. After washing the column with 5–10 bed volumes of the NaCl containing buffer, the column was washed with 5–10 bed volumes of the same buffer containing no NaCl. Adenylate cyclase activity was eluted with a solution containing 25 mM triethanolamine at pH 7.6, 1 mM dithiothreitol and 0.3% or 1.0% Triton X-100. The fractions containing adenylate cyclase activity contained no detectable calmodulin (concentrations of 20 ng/ml would have been detectable). The addition of purified calmodulin in the presence or absence of Ca^{2+} to the apparently calmodulin-deficient but solubilized adenylate cyclase, also resulted in no demonstrable effects on enzyme activity.

DISCUSSION

Guinea pig spermatozoa appear to possess an adenylate cyclase activity that is stimulated 4–5-fold by very low (μM) concentrations of Ca^{2+} in the presence of 5 mM Mg^{2+}. The stimulation by Ca^{2+} is observed with enzyme associated with the particulate fraction of spermatozoa and appears to be lost when the enzyme is solubilized with detergent. Similar to an earlier report (Braun, 1975), higher concentrations of Ca^{2+} (5 mM) also stimulated the sperm adenylate cyclase, but these concentrations are probably not physiologically relevant and were not studied further in this report.

The response of the sperm particulate form of adenylate cyclase to low Ca^{2+} concentrations resembles the response of the adenylate cyclase from brain to Ca^{2+} (Brostrom et al., 1975, 1977), in that the Ca^{2+}-stimulated activity in both cases is inhibited by phenothiazine antipsychotic tranquilizers. These drugs are known to inhibit calmodulin effects (Levin and Weiss, 1976, 1977; Srivastava et al., 1979), but are also known to produce other effects such as membrane stabilization (Seeman, 1972) which may not involve calmodulin. Better evidence for regulation of the sperm adenylate cyclase by calmodulin would be the preparation of calmodulin deficient enzyme with a concomitant loss of Ca^{2+} sensitivity, followed by the subsequent addition of purified calmodulin and the acquisition of Ca^{2+} sensitivity. Successful experiments along these lines have been reported for the detergent-solubilized enzyme from brain (Brostrom et al., 1975, 1977).

In attempts to remove calmodulin, the guinea pig sperm homogenate was washed with EGTA, but such treatment failed to remove the particle associated calmodulin. Previous reports have similarly shown that EGTA treatment of membrane particles does not remove all of the membrane bound calmodulin (Brostrom et al., 1975; Teshima and Kakiuchi, 1978). Detergent treatment of the sperm homogenate resulted in solubilization of adenylate cyclase, but the Mg^{2}-dependent activity decreased from ∿10% to <0.05% of the Mn^{2+}-dependent activity after solubilization. A decrease in Mg^{2+}-dependent activity upon solubilization is not observed with the brain adenylate cyclase unless EGTA is added (Johnson and Sutherland, 1973). In the presence of EGTA, brain Mg^{2+}-dependent activity decreases to <10% of the Mn^{2+}-dependent activity, but can be again increased to normal activities by the addition of Mn^{2+}, Ca^{2+} or Co^{2+} (Johnson and Sutherland, 1973). The sperm adenylate cyclase, therefore, displays markedly different behavior than the brain enzyme upon solubilization.

Despite the failure to observe Ca^{2+} stimulation of the detergent dispersed enzyme, endogenous calmodulin was removed from the detergent dispersed enzyme preparation by Phenyl-Sepharose chromatography. The Mg^{2+}-dependent adenylate cyclase activity remained at <0.05% of the Mn^{2+}-dependent activity and added calmodulin continued to have no effect in the presence or absence of Ca^{2+}.

Whether calmodulin has a functional role in the regulation of sperm adenylate cyclase, remains in question. Low concentrations of Ca^{2+} stimulate the sperm enzyme, but these effects could be directly on the enzyme, or could be mediated by a calcium binding moiety (Mikawa et al., 1978) different from calmodulin. The marked decrease in Mg^{2+} activity relative to Mn^{2+} activity upon detergent solubilization suggests that detergent causes the loss of a factor responsible for the Mg^{2+}-dependent

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activity, or a marked conformational change in the area of the metal activator site(s) on the enzyme. The detergent could also, therefore, prevent expression of calmodulin-adenylate cyclase interaction.

We have recently presented evidence which suggests that a Ca^{2+}-dependent elevation of guinea pig sperm cyclic AMP concentrations precedes or coincides with the acrosome reaction (Hyne and Garbers, 1979). Since adenylate cyclase has been reported to be associated with the acrosome or acrosomal membrane (Herman et al., 1976) and calmodulin has been reported to be exclusively associated with the acrosomal region (Jones et al., 1978) an adenylate cyclase-calmodulin interaction under physiological conditions appears possible. The function of the Ca^{2+}-induced elevation of cyclic AMP in spermatozoa is not clear, but it is possible that Ca^{2+} entry into the sperm cell results in the activation of a number of Ca^{2+}-dependent processes. Activation of these processes could result in a coordinated set of events that could culminate in the acrosome reaction or in other physiological events such as motility activation.

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