Activation of Spermatozoan Adenylate Cyclase by a Low Molecular Weight Factor in Porcine Seminal Plasma*

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The ejaculated porcine spermatozoa were fractionated into the cytosol, membrane, midpiece plus tail (flagella) and head fractions, and their adenylate cyclase activities were measured. About 65% of the total activity was located in the flagella fraction. For all the fractions, Mn2+-dependent adenylate cyclase activity was about 20 times higher than Mg2+-dependent activity, and guanine nucleotides, fluoride, and other reagents tested did not activate adenylate cyclase. The results suggest that the GTP-dependent regulatory subunit is absent in porcine spermatozoa.

The porcine seminal plasma was found to stimulate the adenylate cyclase activity in spermatozoa. The stimulating factor in porcine seminal plasma was partially purified by gel filtration and the molecular weight of the factor appeared to be between 200 and 300.

The partially purified factor is heat stable and is not inactivated by treatment with Pronase, trypsin, phospholipase A2, or D but is inactivated by acid hydrolysis. It is easily soluble in water, partially soluble in methanol, and insoluble in ethanol, ethyl ether, chloroform, or acetone.

The activation of sperm adenylate cyclase by the factor occurred without a time lag. The activating effect was dose-dependent, saturated at high dose, and ascribed to the increase of the maximal velocity (Vmax). The effect of the factor appears to be limited to adenylate cyclase in spermatozoa; the factor activated adenylate cyclase both in porcine and bovine spermatozoa but failed to activate those in other porcine tissues. The factor was shown to activate the enzyme not only in the ejaculated spermatozoa but also in the epididymal sperm. The factor was also found to elevate the cAMP level in the intact porcine spermatozoa. The factor enhanced the motility of corpus and cauda epididymal spermatozoa.

These findings indicate the possibility that this factor initiates the spermatozoan motility upon ejaculation through directly activating adenylate cyclase.

cAMP is suggested to be involved in the regulation of the spermatozoan metabolism and motility (1, 2). For example, the acquisition of the potential for progressive motility during epididymal maturation and the activation of motility at ejaculation and during capacitation are considered to be induced by the elevation of intracellular cAMP level (3-6).

Although spermatozoa have both the adenylate cyclase and the cAMP phosphodiesterase activity, which are involved in the regulation of the intracellular concentration of cAMP, the ratios of the former to the latter were reported to be very low in several mammalian spermatozoa (7, 8) and no first messenger for adenylate cyclase in spermatozoa has been found. Furthermore, it was suggested that the GTP-dependent regulatory subunit, which mediates the effect of NaF, guanine nucleotides, or hormone on adenylate cyclase, is absent in the mammalian sperm adenylate cyclase system (9-13). Thus, the cAMP level in spermatozoa is generally elevated by the use of phosphodiesterase inhibitors such as methylxanthines (1, 14, 15). Recently, Bhatnagar and Amund reported that ATP may serve as the physiological regulator of the sperm motility by inhibiting cyclic nucleotide phosphodiesterase (16).

In this communication, we report the occurrence of an adenylate cyclase system in porcine spermatozoa that is not stimulated by fluoride nor by guanine nucleotides after the existence of a low molecular weight factor in the porcine seminal plasma which stimulates the sperm adenylate cyclase specifically and enhances sperm motility.

MATERIALS AND METHODS

Isolation of Sperm Fractions—Semen was collected from pig and bull using an artificial vagina. Sperm was separated from seminal plasma by layering 15 ml of semen on 20 ml of 1.3 M sucrose, 0.15 M NaCl and centrifuging for 1 h at 55,000 x g in a Beckman SW 28 rotor. The seminal plasma were collected as the supernatant over the sucrose layer. The precipitated sperm was resuspended in 65 ml of 5 mM HEPES, pH 7.0, and homogenized using a glass-Teflon homogenizer. The homogenate was fractionated by the method of Zahler and Doak (17) with slight modifications as follows. 10 ml of sperm homogenate were layered on discontinuous gradients consisting of 8 ml of 1.3 M sucrose, 0.15 M NaCl, 19 ml of 1.7 M sucrose, 0.15 M NaCl, and 10 ml of 2.0 M sucrose, 0.15 M NaCl. The solutions were centrifuged for 3 h at 100,000 x g in Beckman SW 28 rotor. After removing the soluble fraction from the top of each gradient, membrane fractions (plasma membranes and outer acrosomal membranes according to Zahler and Doak (17)) were collected from the sample, 1.3 M sucrose and the 1.3 M, 1.7 M sucrose interface. Midpiece plus tail (flagella) and head fractions were collected from the 1.7 M, 2.0 M sucrose interface and the precipitates, respectively. Each fraction was purified by two more successive discontinuous sucrose gradient centrifugations. Purified fractions were washed twice by centrifugation and were resuspended in 5 mM HEPES, pH 7.0, and were finally stored at 0 °C for the assay of the adenylate cyclase. Microscopic observation of the fractions revealed that contamination is negligible.

Table I shows the characterization and purity of the fractions by the marker enzyme activities. The membrane fractions contained the highest specific activities of 5'-nucleotidase and alkaline phosphatase.

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1 The abbreviations used are: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N',N'-tetraacetic acid; GppNHp, guanylylimidodiphosphate.

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Flagella fraction was characterized by high specific activities of Mg$^{2+}$-dependent ATPase and succinate-cytochrome c reductase and head fraction had a high specific activity of acrosin, respectively. The soluble fraction had a high activity of β-N-acetyl glucosaminidase.

Partial Purification of the Stimulating Factor—About 200 ml of porcine seminal plasma was filtered through Amicon PM-10 ultrafilter membrane. The filtrate was lyophilized to one-twentieth of its original volume and applied on Sephadex G-15 column (2.6 × 90 cm). The column was washed with 30 mM NaCl, 10 mM Tris-HCl, pH 7.4, at 4 °C and the fractions that contain the stimulating factor were collected (Fig. 2a). The fractions were gel filtrated again through the same column under the same conditions. The stimulating factor eluted out was concentrated to 5 ml and placed on Bio-Gel P-2 column (2.6 × 90 cm) followed by washing with 30 mM NaCl, 10 mM Tris-HCl, pH 7.4, at 4 °C (Fig. 2b). The fractions that contain the activity were combined and stored at 0 °C.

Adenylate Cyclase Assay—The adenylate cyclase activity was determined as described previously (18) by incubating 0.6 ml of the standard reaction mixture containing 1 mM ATP, 10 mM theophylline, 5 mM phosphoenolpyruvate, 10 μg of pyruvate kinase, 5 mM MgCl$_2$, 10 mM KCl, 1 mM EDTA, 20 mM Tris-HCl buffer (pH 7.4) and the enzyme (the flagella fraction except when otherwise specified), at 37 °C for a given time. The reaction was stopped by heating at 100 °C for 4 min and cAMP thus formed was assayed by radioimmunoassay using cAMP-radioimmunooassay kits obtained from New England Nuclear. Protein was assayed by the method of Lowry et al. (19) using bovine serum albumin as a standard.

Enzyme Assay—Acrosin, β-N-acetyl glucosaminidase, and alkaline phosphatase activities were measured according to Zahler and Doek (17), 5'-Nucleotidase, Mg$^{2+}$-dependent ATPase, and succinate-cytochrome c reductase assays were assayed according to Michell and Hawthorne (20), Fleischer and Fleischer (21), and Tisdale (22), respectively.

Chemicals—CAMP, ATP, adenosine, GTP, GppNHp, phosphoenolpyruvate, and pyruvate kinase were purchased from Boehringer Mannheim. Theophylline was obtained from the Tokyo Chemical Industry, Japan. Sperm, spermidine, ergothioneine, taurine and ergothionine were purchased from Sigma. Cyclic AMP-radioimmunoassay kit containing $^{32}$P-labeled succinnic acid (1.5 pCi/kit) was obtained from New England Nuclear.

### RESULTS

Localization and Partial Characterization of Porcine Sperm Adenylate Cyclase—Adenylate cyclase activities in the cytosol, membrane, flagella, and head fractions, prepared from porcine spermatozoa as described under "Materials and Methods," were measured using 5 mM Mg$^{2+}$ or Mn$^{2+}$ as the divalent cation. As shown in Table II, the membrane fraction and the flagella fraction had the highest specific activity and approximately 65% of the total activity was found in the flagella fraction in accordance with the results of Bhatnagar et al. (23). The basal activities of these fractions in the presence of Mn$^{2+}$ were almost 20 times higher than those in the presence of Mg$^{2+}$, though the distribution of activity was similar for the two cations. No change in the specific activity was observed when 10 mM NaF, 0.1 mM GTP, or 0.1 mM GppNHP was added to the assay mixture. These results suggest that the GTP-dependent regulatory subunit (G/F) might be absent in the porcine sperm adenylate cyclase system as well as in other mammalian sperm systems.

Various substances were tested for the activating effect on adenylate cyclase in porcine spermatozoa. All of the following were found to be ineffective: prostaglandins (A$_1$, B, E$_1$, E$_2$, F$_1$, and F$_2$), adenosine, isoproterenol, thyroxine, spermine, spermidine, ergothioneine, taurine which has been reported as an essential serum component for the capacitation of hamster spermatozoa (24), and several amino acids abundantly contained in seminal plasma (glycine, alanine, leucine, valine, serine, arginine, lysine, glutamic acid, and aspartic acid) (data are not shown).

The Stimulation of Adenylate Cyclase Activity in Porcine Spermatozoa by a Low Molecular Weight Factor in Seminal Plasma—There has been no report about endogenous substances that regulate the sperm adenylate cyclase activity.

### Table I

Enzymatic characterization of the porcine sperm fractions

<table>
<thead>
<tr>
<th>Fraction</th>
<th>5'-Nucleotidase</th>
<th>Alkaline Phosphatase</th>
<th>Mg$^{2+}$-ATPase</th>
<th>Succinate-cytochrome c Reductase</th>
<th>Acrosin</th>
<th>β-N-Acetyl Glucosaminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>42.0 ± 3.1</td>
<td>0.38 ± 0</td>
<td>59.2 ± 5.0</td>
<td>550 ± 16</td>
<td>0.61 ± 0.1</td>
<td>10.7 ± 1.4</td>
</tr>
<tr>
<td>Cytosol</td>
<td>35.1 ± 2.7</td>
<td>0.15 ± 0</td>
<td>35.1 ± 2.8</td>
<td>59.7 ± 7.0</td>
<td>0.85 ± 0.1</td>
<td>10.4 ± 0.3</td>
</tr>
<tr>
<td>Membranes</td>
<td>192 ± 8.5</td>
<td>1.00 ± 0.1</td>
<td>90.1 ± 4.2</td>
<td>812 ± 12</td>
<td>0.71 ± 0.1</td>
<td>5.18 ± 1.1</td>
</tr>
<tr>
<td>Midpiece plus</td>
<td>34.3 ± 0.9</td>
<td>0.30 ± 0</td>
<td>25.8 ± 3.1</td>
<td>199 ± 13</td>
<td>1.19 ± 0.2</td>
<td>7.76 ± 0.9</td>
</tr>
<tr>
<td>Head</td>
<td>53.8 ± 2.1</td>
<td>0.17 ± 0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table II

Distribution of adenylate cyclase activities in the sperm fractions and the effects of NaF, GTP, GppNHP, and porcine seminal plasma

Sperm fractions equivalent to 80-130 μg of protein were incubated at 37 °C for 20 min in the standard reaction mixture containing either 5 mM MnCl$_2$ or 5 mM MgCl$_2$ as the divalent cation. Data are expressed as mean ± S.E. from 3 experiments, each with duplicate determinations.

<table>
<thead>
<tr>
<th>Sperm fraction</th>
<th>Mn$^{2+}$ Total activity (pmol/μg protein/30 min)</th>
<th>Mg$^{2+}$ Total activity (pmol/μg protein/30 min)</th>
<th>NaF (10 μM NaF)</th>
<th>GTP (0.1 mM GTP)</th>
<th>GppNHP (0.1 mM GppNHP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>661 ± 22</td>
<td>24.7 ± 3</td>
<td>9</td>
<td>25.1 ± 1</td>
<td>23.3 ± 6</td>
</tr>
<tr>
<td>Membranes</td>
<td>2930 ± 58</td>
<td>120 ± 23</td>
<td>8</td>
<td>126 ± 5</td>
<td>125 ± 8</td>
</tr>
<tr>
<td>Midpiece plus</td>
<td>2790 ± 110</td>
<td>117 ± 13</td>
<td>64</td>
<td>130 ± 6</td>
<td>111 ± 14</td>
</tr>
<tr>
<td>Head</td>
<td>411 ± 32</td>
<td>17.5 ± 2</td>
<td>19</td>
<td>449 ± 27</td>
<td>63.7 ± 8</td>
</tr>
</tbody>
</table>

*Measured in fmol of cAMP/μg of protein/20 min.
During the course of this study, we found that some substance(s) in porcine seminal plasma evidently stimulates the enzyme activity. As shown in Table II, addition of seminal plasma from fresh porcine semen to the assay mixture stimulated the Mg ++-dependent adenylate cyclase activity in each of the fractions up to 4 times over the basal activity. On the other hand, the Mn ++-dependent activity is scarcely stimulated by seminal plasma. The authenticity of the product formed by spermatozoan adenylate cyclase in response to seminal plasma was verified as follows. The reaction was carried out by using [2,8-3H]ATP as a substrate under the same assay conditions as described under “Materials and Methods,” except that 1 mM of cold cAMP was contained in the assay mixture. [3H]cAMP formed by the enzymatic reaction was identified by the polyethyleneimine cellulose thin layer chromatography using 0.3 M LiCl as solvents. Under these conditions, the Rp values for ATP, ADP, AMP, and cAMP were 0.02, 0.04, 0.22, and 0.50, respectively. The porcine seminal plasma added to the reaction mixture increased the radioactivities in the spot of cAMP at the same rate as that determined by the radiomunnoassay. These results confirmed that the porcine seminal plasma actually increased the formation of cAMP by adenylate cyclase in the porcine spermatozoa. Fig. 1 shows some properties of the stimulating factor in seminal plasma, using flagella fraction as a source of adenylate cyclase. First, this stimulatory effect was dose-dependent and was saturated at high dose. The stimulating factor was heat stable and was inactivated by dialysis or acid hydrolysis and was resistant against the digestion by Pronase, trypsin, phospholipase A2 or D. The chelating agents such as EGTA and EDTA inhibited slightly adenylate cyclase activity itself probably by lowering divalent cations in the assay mixture, but the degree of the activation by the factor was not influenced by these reagents. GTP and NaF had no effect on the enzyme activity stimulated by the factor.

The activating factor was partially purified by gel filtration through Sephadex G-15 (Fig. 2a) and Bio-Gel P-2 (Fig. 2b) columns as described under “Materials and Methods.” The molecular weight of this factor seems to be between 200 and 300 as the factor was eluted from Bio-Gel P-2 column just after the reduced form of glutathione. The partially purified factor was easily soluble in water and partially soluble in methanol, but insoluble in ethanol, ethyl ether, chloroform, or acetone.

The stimulation of cAMP formation by this factor is not due to the inhibition of cAMP phosphodiesterase, because the factor inhibits neither the cAMP phosphodiesterase activity in porcine sperm homogenates nor that purified from porcine brain (data are not shown). Theophylline, an effective inhibitor of phosphodiesterase, has been reported to inhibit ade-
Activating Factor of Sperm Adenylyl Cyclase

In some cases (25–27), the adenylyl cyclase activity using [2,8-3H]ATP as substrate in the presence of high concentrations of cold cAMP (1 mM), 10 mM of theophylline inhibited only slightly (15% inhibition) the adenylyl cyclase activity of the flagella fractions. The factor stimulated the cAMP formation about 150% in the presence of theophylline, suggesting that the effect of the factor is not due to the relief of the theophylline inhibition of the enzyme.

The Analysis of the Stimulation of Adenylyl Cyclase Activity by the Partially Purified Factor—In order to ascertain the linearity of CAMP formation with increase in enzyme concentration the experiment shown in Fig. 3 was performed. It is clear that both in the absence and presence of the partially purified activating factor the product formation is directly proportional to enzyme concentration. The conditions used in Fig. 3 were identical with those used in subsequent experiments.

Kinetics of cAMP formation by the flagella fractions of porcine spermatozoa were investigated in the presence and absence of the factor. As shown in Fig. 4, the cAMP formation proceeded linearly up to 20 min, and the stimulation in the adenylyl cyclase activity by the factor was observed clearly even 1 min after the initiation of the incubation. Therefore, it is thought that the stimulatory effect arises from a direct interaction with adenylyl cyclase system in spermatozoa.

The effects of the factor on the kinetic parameters of the sperm adenylyl cyclase activity were investigated. In this experiment, the concentrations of MgCl₂ in the standard reaction mixture were raised to 20 mM to keep the free metal ion concentration constant. Fig. 5 shows the initial velocity of adenylyl cyclase reactions as a function of the substrate ATP concentrations in the presence and absence of the factor. The kinetic parameters such as $K_m$ and $V_{max}$ were obtained from the Lineweaver-Burk plots (the upper inset of Fig. 5). $K_m$ and $V_{max}$ in the absence of the factor were 0.65 mM ATP and 8.8 fmol/µg of protein/min, respectively. Clearly, the stimulation by the factor is due to an increase in $V_{max}$.

Fig. 6 shows the effect of the factor on the adenylyl cyclase systems from several tissues. The particulate fractions, prepared from porcine brain, liver, muscle, adipose tissue, and

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**Fig. 3.** Dependence of cAMP formation on the concentrations of the enzyme protein and the effect of the partially purified factor. The flagella fractions at various concentrations were incubated at 37 °C for 20 min in the standard reaction mixture containing ATP at various concentrations. The concentrations of MgCl₂ in the reaction mixture were 20 mM. ○, ●, and □, indicate the addition of none, 50 µl, and 100 µl of the partially purified factor, respectively. The upper inset shows the Lineweaver-Burk plots of the reaction rates. Each point in the figure is mean ± S.E. from 2 experiments, each with triplicate determinations.

**Fig. 4.** Kinetics of cAMP formation by the flagella fraction of porcine spermatozoa and the effect of the partially purified factor. The flagella fraction equivalent to 110 µg of protein was incubated at 37 °C for various intervals of time in the standard reaction mixture with (●) or without (○) 200 µl of the partially purified factor. Each point in the figure is a mean of the duplicate determinations.

**Fig. 5.** Effect of the partially purified factor on the kinetic parameters of adenylyl cyclase of the porcine sperm flagella fraction. The flagella fraction equivalent to 75 µg of protein was incubated at 37 °C for 10 min in the standard reaction mixture containing ATP at various concentrations. The concentrations of MgCl₂ in the reaction mixture were 20 mM. ○, ●, and □, indicate the addition of none, 50 µl, and 100 µl of the partially purified factor, respectively. The upper inset shows the Lineweaver-Burk plots of the reaction rates. Each point in the figure is mean ± S.E. from 2 experiments, each with triplicate determinations.
porcine and bovine spermatozoa, were assayed for the adenylate cyclase activity in the presence and absence of the factor. The factor stimulated specifically spermatozoan adenylate cyclases but not those in other tissues. The effect of the factor on the adenylate cyclase activity in epididymal spermatozoa was also investigated. Table III shows the activity of the enzymes from caput, corpus, and cauda epididymal spermatozoa and the ejaculated spermatozoa. Epididymal and ejaculated spermatozoa were shown to have similar basal activities and sensitivity to the factor, except the slightly lower figures for the caput epididymal spermatozoa.

The activation of adenylate cyclase by the factor was found not only in the broken cell preparations but also in the intact spermatozoa. The washed sperm was incubated in Ca²⁺-free Krebs-Ringer phosphate buffer with or without the factor at 37 °C, and after various intervals of time aliquots of the incubation mixture were pipetted out and were added to ice-cold 6% trichloroacetic acid. After quick homogenization by sonication, their cAMP contents were measured by radioimmunoassay. As shown in Fig. 7, the factor elevated the intracellular cAMP level slightly (about 20%) over the basal one. Addition of 10 mM theophylline to the incubation mixture increased the basal cAMP level slightly, probably because of the inhibition of cAMP phosphodiesterase. In the presence of theophylline, the marked elevation of cAMP level by the factor was observed.

**DISCUSSION**

The hormone-sensitive adenylate cyclase systems in many tissues contain at least three components, namely hormone receptor, catalytic subunit, and one of two kinds of GTP-dependent regulatory subunit which transmits either the stimulatory (Ns or G/F) or the inhibitory (Ni) signal from the hormone receptor to the catalytic subunit (28). But, in several mammalian spermatozoa, the adenylate cyclase system lacks the GTP-dependent regulatory subunit (9–13). Various substances such as hormones, guanine nucleotides, fluoride, and calmodulin have been tested and found not to activate the sperm adenylate cyclase. It was reported that thyroxine and triiodothyronine slightly increased the cAMP level in monkey sperm (29), but Herman et al. showed that thyroxine did not activate adenylate cyclase in bovine sperm (10), which coincides with our observation in porcine spermatozoa.

The enhancement of the intracellular cAMP level has been shown to stimulate and maintain the motility of various spermatozoa (1–6) and demembranated sperm models (30),

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**TABLE III**

**Effects of the partially purified factor on the adenylate cyclase activity in epididymal spermatozoa**

<table>
<thead>
<tr>
<th>Sperm origin</th>
<th>Adenylate cyclase activity (fMol cAMP/pg protein/20 min)</th>
<th>Factor stimulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caput epididymis</td>
<td>50.5 ± 4</td>
<td>141</td>
</tr>
<tr>
<td>Corpus epididymis</td>
<td>115 ± 6</td>
<td>288</td>
</tr>
<tr>
<td>Cauda epididymis</td>
<td>98.0 ± 10</td>
<td>306</td>
</tr>
<tr>
<td>Ejaculated semen</td>
<td>81.8 ± 7</td>
<td>272</td>
</tr>
</tbody>
</table>

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**FIG. 7.** Effect of the partially purified factor on the cAMP level in porcine spermatozoa. The ejaculated porcine spermatozoa were washed with Ca²⁺-free Krebs-Ringer phosphate buffer by centrifugation and then suspended in the same buffer. The washed spermatozoa (final 5 x 10⁵ cells/ml) were incubated in 1 ml of Ca²⁺-free Krebs-Ringer phosphate buffer with no additions (O), 10 mM theophylline (D), 200 µl of the partially purified factor (O), or 10 mM theophylline plus 200 µl of the partially purified factor (A), at 37 °C. After various intervals of time, 50 µl of aliquots of the incubation mixture were pipetted out, added to 1 ml of ice-cold 6% trichloroacetic acid, and quickly homogenized by sonication. The homogenate was centrifuged at 3000 rpm for 15 min, and the supernatant was collected for cAMP assay. After trichloroacetic acid was removed from the supernatant, the cAMP content was determined as described under "Materials and Methods." The data are expressed as a mean ± deviation from duplicate determinations.
although the mechanisms involved in the regulation of cAMP metabolism in the spermatozoa remain uncertain. Bhatnagar and Anand suggested that the elevation of cAMP level may be caused by the inhibition of cAMP phosphodiesterase (15). It has also been reported that the elevation of the cAMP level by calcium supplied at ejaculation (31) or by dilution of the highly concentrated cauda epididymal semen by accessory sex gland fluids during ejaculation (4) may produce the sperm motility.

We confirmed that porcine sperm as well as other mammalian spermatozoa had adenylate cyclase, which was located largely in flagella. But neither guanine nucleotides nor fluoride were shown to activate adenylate cyclase, which suggests that the enzyme lacks the GTP-dependent regulatory subunit.

We tried to seek an exogenous substance that could affect directly the adenylate cyclase activity in spermatozoa and found that a low molecular weight substance(s) in porcine seminal plasma markedly stimulates the accumulation of cAMP in spermatozoa. This stimulatory effect is thought not due to the inhibition of cAMP phosphodiesterase but to the activation of adenylate cyclase. The substrate for adenylate cyclase (ATP) in seminal plasma, if any, is not responsible for the observed activation of adenylate cyclase. The concentrations of ATP in the fraction of the partially purified factor estimated from the absorbance at 260 nm are below 5 μM.

The factor was shown to activate the adenylate cyclase in spermatozoa not only of the porcine but also of the bovine origin though it could not activate the enzymes in other tissues (Fig. 6). Although the reason for the tissue specificity of this factor is unknown, one possible explanation is that only the spermatozoan adenylate cyclase system has a specific binding site for the factor. Another explanation is that the environment of adenylate cyclase in spermatozoa is different from that in the other tissues, and the factor may be able to interact with the catalytic subunit without interacting with the GTP-dependent regulatory subunit. In this connection, it is interesting that adenylate cyclase is stimulated by the factor in the presence of Mg2+ but not in the presence of Mn2+.

Although this factor has not yet been identified, this is the first observation that mammalian sperm adenylate cyclase is stimulated by an endogenous substance other than substrate or divalent cations without the aid of the GTP-dependent regulatory subunit. Garbers and Hardman showed that the enzyme lacks the GTP-dependent regulatory subunit. Garbers, D. L., Lust, N. L., Gorman, S. K., and Lardy, H. A. (1973) Biochem. Biophys. Res. Commun. 48, 1331-1338.

Another factor was a low molecular weight (approximately 1800) substance and elevated the concentrations of both the cAMP and the cGMP in Ca2+-free sea water (37). The factor in porcine seminal plasma doesn’t require Ca2+ to stimulate adenylate cyclase and has smaller molecular weight than the egg factors.

Porcine spermatozoa in all regions of epididymis are immobile in epididymal fluids, but they develop the potential for motility by the time they reach the cauda epididymis (38). Although the cauda epididymal immobilized spermatozoa develop partial motility after dilution of the epididymal fluids (38), they finally initiate the coordinated movement only after ejaculation. The actual initiation of sperm motility upon ejaculation may be induced by the action of this factor in seminal plasma through activating adenylate cyclase and elevating the intracellular cAMP level.2 In fact, the factor was shown to activate adenylate cyclase not only in the ejaculated spermatozoa but also in the epididymal sperm. Furthermore it was also found to enhance the accumulation of the intracellular cAMP when added to the intact spermatozoa extracellularly (Fig. 7). Further studies to define the role of the factor in sperm motility are in progress.

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REFERENCES


2 In preliminary experiments, it was found that the factor enhanced the motility of the corpus and cauda epididymal spermatozoa to the same extent as 10 mM theophylline. When ejaculated spermatozoa were washed with Ca2+-free Krebs-Ringer phosphate buffer the motility decreased and was restored by the factor. In contrast, caput epididymal sperm, which are not motile, were not affected by the factor suggesting that spermatozoa in this region have not yet developed the potential for motility.
Activating Factor of Sperm Adenylate Cyclase

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