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Stimulation of Sperm Respiration Rates by Speract and Resact at Alkaline Extracellular pH

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ABSTRACT

At an extracellular pH of 6.6, a peptide (resact) isolated from the egg jelly of *Arbacia punctulata* increased the respiration rates of *A. punctulata* spermatozoa but did not activate sperm cells from *Lytechinus pictus*. In contrast, speract (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly), elevated the respiration rates of *L. pictus* but not *A. punctulata* spermatozoa. At normal seawater pH (7.6–8.0) egg jelly from *A. punctulata*, or egg jelly from *L. pictus* purified free of speract, inhibited *L. pictus* sperm respiration rates. Similarly, the egg jelly from *L. pictus* inhibited the respiration rates of *A. punctulata* spermatozoa. The jelly component responsible for the inhibition of respiration was nondialyzable. The inhibition of respiration induced by jelly could be reversed by the addition of speract to *L. pictus* spermatozoa and by the addition of resact to *A. punctulata* spermatozoa. Speract stimulated *L. pictus* sperm respiration half-maximally at about 1 nM in the presence of either heterologous or homologous (speract-free) jelly. Monensin A, an ionophore which elevates sperm intracellular pH, reversed the jelly inhibition of respiration. These results demonstrate that two peptides associated with eggs (speract and resact) can stimulate sperm motility and metabolism in the face of inhibitory components present in the egg jelly. Additionally, the peptides demonstrate species specificity.

INTRODUCTION

The egg jelly of sea urchins appears to contain factor(s) which are important for the normal fertilization of sea urchins (Lillie, 113; Dan, 1952). One factor, a fucose-sulfate-rich polymer, has been shown to induce the acrosome reaction of homologous spermatozoa (SeGall and Lennarz, 1979; Kopf and Garbers, 1981); it also causes a rapid accumulation of $^{45}Ca^{2+}$ and marked elevations of cyclic AMP (Kopf and Garbers, 1981). Another factor, whose function during fertilization has not been determined, has been isolated from the egg jelly of the sea urchins, *Strongylocentrotus purpuratus* and *Hemicentrotus pulcherrimus;* it has been named speract and is a decapeptide (Hansbrough and Garbers, 1981; Suzuki et al., 1981; Garbers et al., 1982). Speract has the amino acid sequence of Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly. A primary effect of the peptide appears to be the induction of a net H^+ efflux from the sperm cell with a resultant alkalinization of intracellular pH (Repaske and Garbers, 1983).

Speract, and other peptides associated with *H. pulcherrimus* and *Anthocidaris crassispina* eggs, have been shown to markedly stimulate the respiration of sea urchin spermatozoa at acidic, but to only marginally stimulate at alkaline extracellular pH values (Hansbrough and Garbers, 1981; Suzuki et al., 1981; Garbers et al., 1982; Nomura et al., 1983). Since seawater pH is alkaline, the physiological significance of the larger stimulation at acidic pH has not been clear, although it has been suggested that the pH of egg jelly is acidic (Vasseur and Hagstrom, 1946; Ohtake, 1976), because of its high content of carboxyl and ester-linked sulfate residues (SeGall and Lennarz, 1979; Isaka et al., 1970; Ishihara et al., 1973); it has been compared to a polyelectrolyte gel (Levin et al., 1964; Goldstein et al., 1964). Recently, Holland and Cross (1983), however, have suggested that the pH of egg jelly is actually in the range of 7.99 ± 0.035.

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The identification of the actual functional role of various jelly components is now possible because of their purification. Here, we report that both homologous egg jelly free of speract and heterologous egg jelly inhibit sperm respiration rates at either acidic or alkaline extracellular pH. The subsequent addition of speract or resact results in relief of the jelly-induced respiratory inhibition. Speract is specific for *L. pictus* while resact is specific for *A. punctulata.* Monensin A, an ionophore which mimics the effects of speract at acidic pH values, also reverses the inhibition of respiration by egg jelly.

MATERIALS AND METHODS

Materials

A. punctulata were obtained from the Marine Biological Laboratory, Woods Hole, MA and *L. pictus* were purchased from Pacific Bio-Marine, Venice, CA. Monensin A was a generous gift from Dr. Robert Hamill, Eli Lilly and Co., Indianapolis, IN. Speract (Gly-Phe-Asp-Leu-Asn-Gly-Gly-Gly-Val-Gly) was custom synthesized by Peninsula Labs., Inc., San Carlos, CA.

Gamete Collection and Incubation

Spermatozoa or eggs were obtained after the injection of sea urchins with 0.5 M KCl. Spermatozoa were collected "dry" at 5°C and stored on ice at approximately 400 mg (wet weight)/ml until use. In general, spermatozoa were incubated in artificial sea water in the presence or absence of the egg jelly. All incubations were carried out at 20°C in artificial sea water (ASW) composed of 454 mM NaCl, 9.7 mM KCl, 24.9 mM $MgCl_2$, 9.6 mM $CaCl_2$, 27.1 mM $MgSO_4$, 4.4 mM $NaHCO_3$ and 10 mM Tris or 10 mM Tris-10 mM N-[2-acetamido]-2-aminoethane sulfonic acid (ACES). ASW which did not contain Tris or ACES was used in some experiments. Unless otherwise indicated, the reactions were initiated by the addition of spermatozoa.

Determination of Respiration Rates

Respiration rates were determined using a Gilson K-IC Table Top Oxygraph equipped with a 2.2-ml capacity temperature control chamber fitted with a Clark type electrode and a semimicro pH electrode (Radiometer GK-2421C).

Immediately prior to use, one volume of dry spermatozoa was suspended in three volumes of the same ASW as used in the incubation; in some cases dry sperm were used without dilution. The sperm suspension (10 mg wet weight/100 μl) or dry sperm (10 mg wet weight/25 μl) was added to 2.1 ml or 2.2 ml of ASW or egg jelly solution. After recording stable basal respiration rates for several minutes, various agents were added. The new respiration rates were determined over the next 3 min. Respiration rates were determined assuming the solubility of pure O_2 at 1 atmosphere to be 0.0310 ml/ml of ASW at 20°C (Umbreit, 1964).

Isolation of Jelly Coat by Mechanical Means

In some experiments, eggs were collected in nonbuffered ASW (pH 7.5–7.9) and allowed to stand at 5°C with occasional stirring for 5–6 min. The suspension (10% v/v) was then gently pipetted up and down through a wide-base disposable plastic pipet. After this, the eggs were centrifuged at 4°C at 1500 × *g* for 10 min. The supernatant fluid containing the jelly coat was centrifuged at 10,000 × *g* for 60 min at 4°C and the resulting supernatant fluid was used as the egg jelly solution.

Isolation of Jelly Coat by Acid Treatment

After injection of 0.5 M KCl into the coelomic cavity of sea urchins, eggs were collected in buffered ASW (pH 7.8–8.0). The eggs were centrifuged at 1500 × *g* for 10 min at 5°C. The pelleted eggs were suspended (10% v/v) in ASW buffered to pH 5.5 with 10 mM Tris-10 mM ACES. After 5–6 min with occasional gentle stirring, the eggs were centrifuged at 1500 × *g* for 10 min at 5°C. The supernatant fluid was centrifuged at 10,000 × *g* for 60 min at 4°C. The pH of the resultant supernatant fluid was adjusted to 7.5 by the addition of 1 N NaOH.

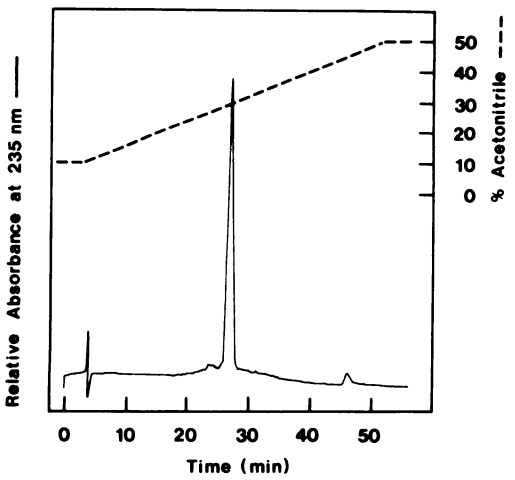

FIG. 1. High-pressure liquid chromatography (HPLC) of the purified *A. punctulata* peptide (resact). Resact was extracted from *A. punctulata* egg jelly with 70% ethanol and purified by chromatography on a Sephadex G-10 column, followed by HPLC on an octyl column (4.6 × 250 mm). Fractions containing activity obtained from the HPLC column were pooled and lyophilized. The residue, which contained approximately 100 nmol of resact, was applied to the octyl column (4.6 × 250 mm) equilibrated with 10% acetonitrile in 0.1% trifluoroacetic acid. A program with a constant flow rate of 1.0 ml/min at 40°C was initiated at 2 min to give the linear acetonitrile concentration shown in the Figure. The column effluent was monitored for absorbance at 235 nm and the peak fractions (27–28 min) were pooled and used for experiments.

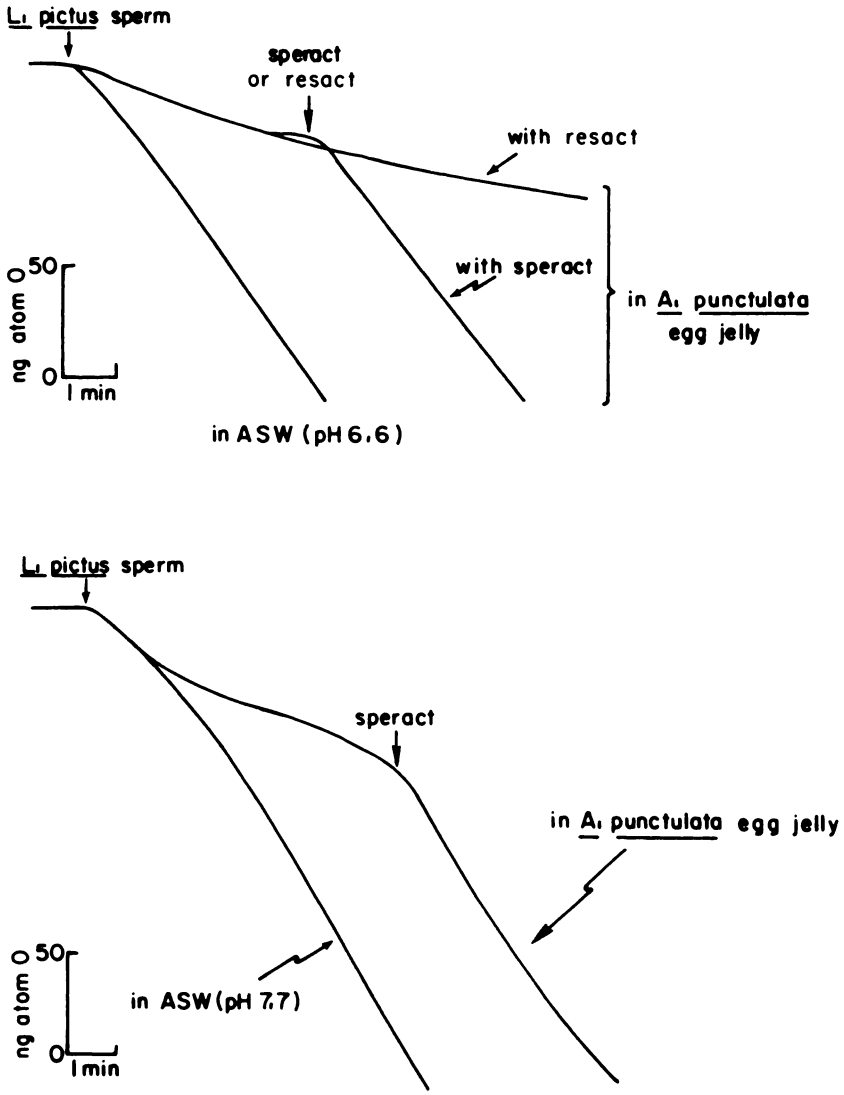

FIG. 2. Typical oxygraph traces of *L. pictus* sperm respiration in the presence or absence of *A. punctulata* egg jelly at acidic or alkaline pH. *Top)* Respiration rates at pH 6.6. *A. punctulata* eggs were collected in buffered ASW and the egg jelly was then prepared by acid treatment as described in the text. The egg jelly was adjusted to pH 6.6 by the addition of 1 N HCl and was added to give 4.0 mg/ml protein and 63 nmol/ml fucose. *Bottom)* Respiration rates at pH 7.7. *A. punctulata* eggs were collected in nonbuffered ASW and the egg jelly was prepared by mechanical means as described in the text. The egg jelly was added to give 4.1 mg/ml protein and 55 nmol/ml fucose. Sperm respiration was determined at 20°C. At the points indicated, spermatozoa, speract or resact were added to give final concentrations of approximately 4.5 mg (wet weight)/ml, 1.0 nM or 200 nM, respectively.

Charcoal Treatment of Egg Jelly

Since the egg jelly of *L. pictus* contains peptide(s) which stimulate the respiration and motility of the homologous spermatozoa, the egg jelly was treated with activated charcoal to remove the peptides (Hansbrough and Garbers, 1981). Before the treatment, the charcoal was washed extensively with millipore-filtered H_2O and preequilibrated with ASW (pH 7.5). About 40 g of charcoal suspension was added to 90 ml of egg jelly and stirred for 12 h at 5°C. Charcoal was removed by centrifugation at 10,000 × *g* for 30 min and subsequent filtration on Whatman No. 1 filter paper. The filtrate was then dialyzed at 5°C against ASW (pH 8.0) for 12 h. The nondialyzable fraction was centrifuged at 4°C at 10,000 × *g* for 60 min, and the supernatant fluid was subsequently used for experiments.

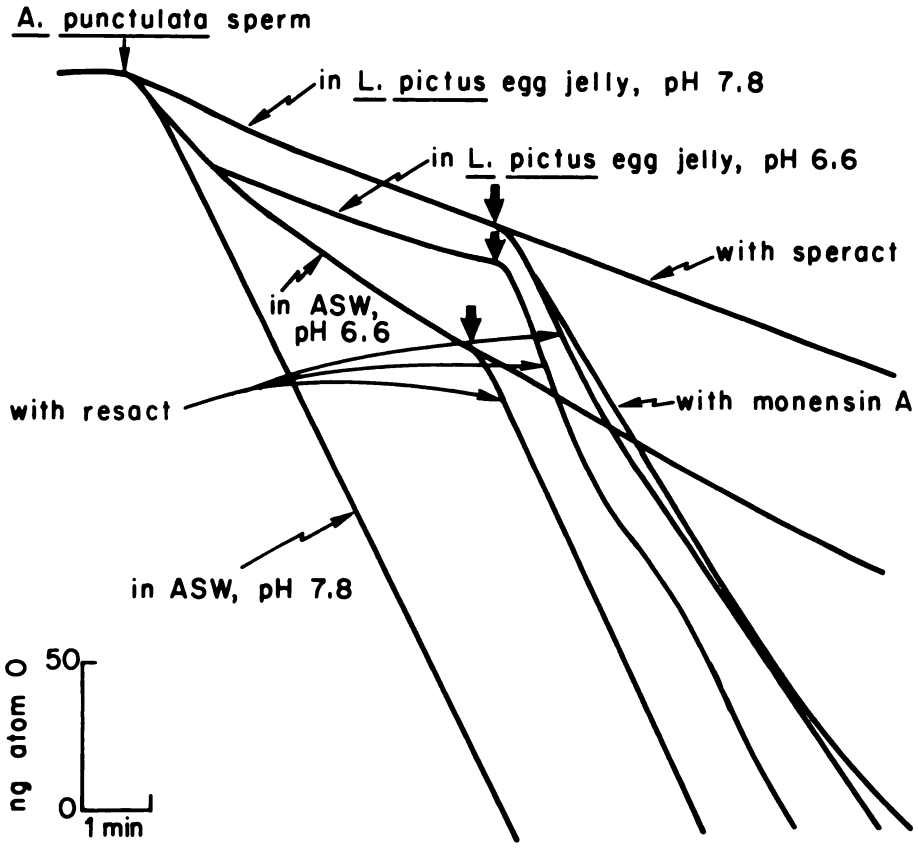

FIG. 3. Typical oxygraph traces of *A. punctulata* sperm respiration in the presence of *L. pictus* egg jelly at pH values of 6.6 or 7.8. *L. pictus* egg jelly was prepared by acid treatment and purified through the charcoal step as described in the text. The egg jelly used was added to give 640 μg/ml protein and 799 nmol/ml fucose and was adjusted to a pH of 6.6 or 7.8. At the indicated points, spermatozoa, speract, resact or monensin A were added to give final concentrations of approximately 4.5 mg (wet weight)/ml, 1.0 nM, 200 nM or 25 μM, respectively.

Other Methods

Protein was determined according to Lowry et al. (1951). Fucose was assayed by the method of Dische and Shettles (1951). Amino acid analysis was as follows: peptide was hydrolyzed in 6 N HCl at 110°C under vaccum for 24 h. The sample was then dried and subsequently analyzed with an LKB 4400 amino acid analyzer. A lack of aromatic amino acids in resact was also confirmed by the U.V. spectrum of the peptide (no absorption maxima in the 250- to 290-nm range was detected.

RESULTS

It has been reported that *A. punctulata* egg jelly lacks the agent capable of stimulating the respiration of *L. variegatus* spermatozoa (Hathaway, 1963) despite a similarity in the gross chemical composition of the egg jelly of both species (SeGall and Lennarz, 1979). Initially, we determined that speract did not activate the respiration rates of *A. punctulata* spermatozoa at acidic pH values. We then determined that the egg jelly from *A. punctulata* contained a substance(s) which stimulated the respiration of its own spermatozoa at pH 6.6 (not shown). This substance did not cross-react with *S. purpuratus* or *L. pictus* spermatozoa. Purification of the substance by high-pressure liquid chromatography demonstrated that it was a peptide (Fig. 1). The amino acid sequence of the peptide has not been determined but its composition is: Thr (0.9 nmol), Pro (0.8 nmol), Gly (4.6 nmol), Ala (0.8 nmol), Val (1.8 nmol), Leu (1.0 nmol), CmCys (2.2 nmol) and Arg (1.0 nmol). The peptide, obviously different from speract based on both amino acid composition as well as biological activity, has been named resact.

Since egg jelly has been shown to inhibit sperm respiration under conditions of normal

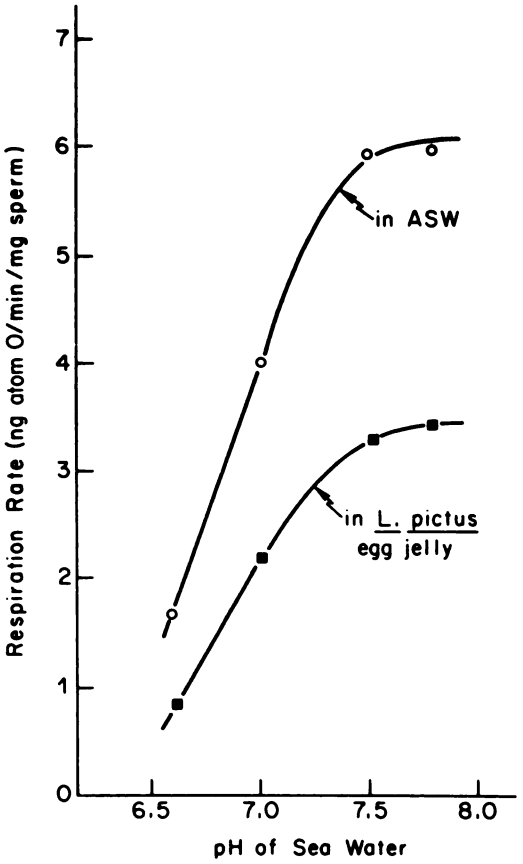

FIG. 4. Inhibition of *A. punctulata* sperm respiration by *L. pictus* egg jelly as a function of extracellular pH. *L. pictus* egg jelly was prepared as described in the legend to Fig. 3. Respiration rates of *A. punctulata* spermatozoa (4.5 mg wet weight/ml) were determined as described in the text in the presence of either *L. pictus* egg jelly or ASW buffered with 10 mM Tris-10 mM ACES to the indicated pH values. *L. pictus* egg jelly was added to give 640 μg/ml protein and 799 nmol/ml fucose.

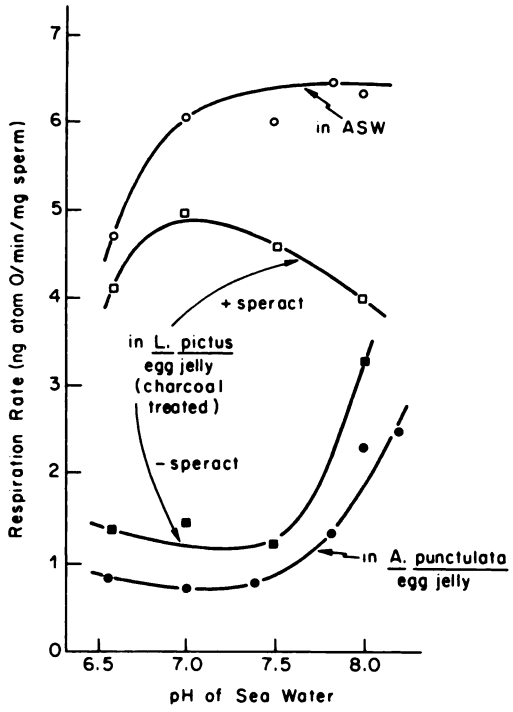

FIG. 5. Inhibition of *L. pictus* sperm respiration rates by homologous (speract-free) (■—■) or heterologous (●—●) jelly at various extracellular pH values and the relief of inhibition by speract. *L. pictus* egg jelly was prepared as described in the legend to Fig. 3 and was added to give 640 μg/ml protein and 799 nmol/ml fucose. *A. punctulata* egg jelly was prepared as described in the legend to Fig. 2 and was added to give 4.0 mg/ml protein and 63 nmol/ml fucose. Respiration rates of *L. pictus* spermatozoa (4.5 mg wet weight/ml) were determined as described in the text at the indicated pH in the presence or absence of egg jelly and in the presence or absence of added speract (final concentration of 3.4 nM).

extracellular pH (Kinsey et al., 1979; Christen et al., 1983), we first tesetd whether or not heterologous egg jelly would inhibit *L. pictus* sperm respiration at acidic (6.6) or alkaline (7.7) pH values. These experiments were now possible because the peptides capable of activating sperm respiration did not cross-react between these sea urchin species. *A. punctulata* jelly effectively inhibited sperm respiration rates at either pH (Fig. 2). The subsequent addition of speract greatly stimulated respiration, whereas the addition of resact did not stimulate at either pH. It should be noted that at pH 6.6, *L. pictus* spermatozoa respire at rates comparable to the rates obtained at pH 7.7.

When *A. punctulata* spermatozoa were inhibited by *L. pictus* egg jelly the same effects were observed except speract did not stimulate the respiration rates whereas resact did stimulate them (Fig. 3). At pH 6.6, *A. punctulata* spermatozoa respired at lower rates than at pH 7.8; this is similar to the situation with *S. purpuratus* spermatozoa (Hansbrough and Garbers, 1981). Monensin A, an ionophore which catalyzes an electroneutral Na^+/H^+ exchange, reproduced the effects of resact on respiration (Fig. 3). When *L. pictus* sperm cells were inhibited by jelly, monensin A also stimulated respiration rates (not shown).

The respiratory inhibition by heterologous egg jelly was dependent upon the extracellular

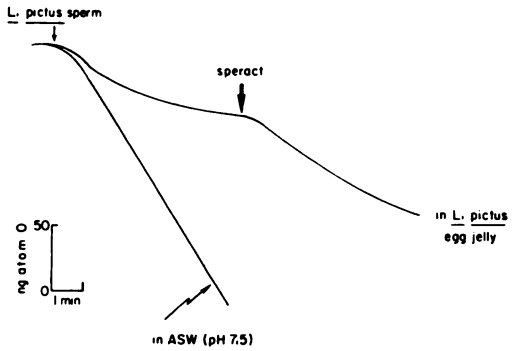

FIG. 6. Typical oxygraph traces of *L. pictus* spermatozoa in the presence or absence of *L. pictus* egg jelly. *L. pictus* egg jelly was prepared by acid treatment and purified through the charcoal step as described in the legend to Fig. 3. The egg jelly was added to give 640 μg/ml protein and 799 nmol/ml fucose. At the points indicated, *L. pictus* spermatozoa and speract were added to give final concentrations of 4.5 mg (wet weight)/ml and 1 nM, respectively.

pH (Figs. 4 and 5), however, the addition of the homologous peptide reversed the inhibition at all pH values, including those at physiological values. The acrosome reaction was not observed with heterologous egg jelly, and the subsequent addition of peptide also did not cause an induction of the acrosome reaction.

After treatment of homologous egg jelly with charcoal to remove speract (Kopf and Garbers, 1981), it inhibited sperm respiration, and the subsequent addition of speract resulted in a stimulation of respiration at all pH values tested (Fig. 5). It was frequently observed, however, that the stimulatory effect of speract in the homologous system was transient, lasting only a few minutes (Fig. 6). This contrasts to the situation in the heterologous system where the respiratory stimulation caused by speract continued during the time course of the incubation (see Figs. 2 and 3). Possibly these differences are due to the occurrence of an acrosome reaction at pH 8.0 since about 70% of the spermatozoa demonstrated an acrosome reaction in the homologous system. This was not investigated further, however, it also should be noted that 3.4 nM speract did not completely reverse the jelly-induced inhibition of respiration (Fig. 5).

The factor responsible for the inhibition of respiration was not dialyzable (not shown). Approximately 700 nmol/ml of added fucose (*L. pictus* jelly) inhibited the respiration of *L.*

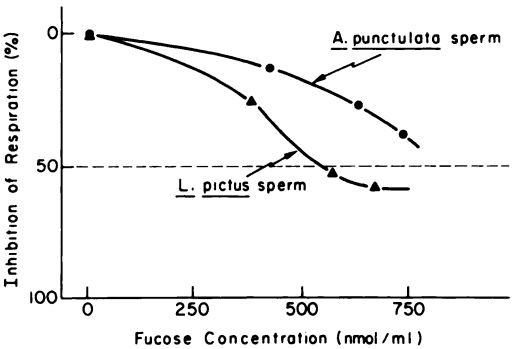

FIG. 7. The inhibition of sperm respiration by *L. pictus* egg jelly as a function of the amount of fucose added. *L. pictus* egg jelly was prepared as described in the legend to Fig. 3 and was added to give the indicated final concentration of fucose. Respiration rates of *L. pictus* or *A. punctulata* spermatozoa (4.5 mg wet weight/ml) were determined as described in the text in the absence or presence of *L. pictus* egg jelly at pH 7.5.

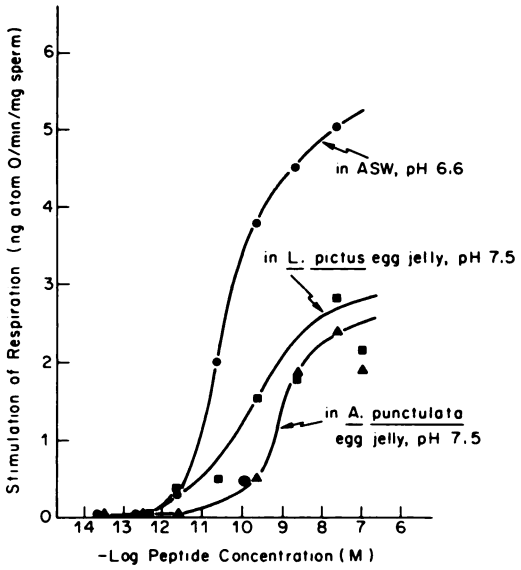

FIG. 8. The stimulation of jelly-inhibited respiration of *L. pictus* spermatozoa by the addition of various concentrations of speract. *A. punctulata* egg jelly or *L. pictus* egg jelly were prepared and added as described in the legends to Fig. 2 and Fig. 3, respectively. The final concentrations of fucose were 63 nmol/ml and 799 nmol/ml for the added *A. punctulata* and *L. pictus* egg jelly, respectively. The stimulation of respiration represents the difference in respiration rates before and after peptide addition and is plotted as a function of −log speract concentration.

pictus spermatozoa by 60% and *A. punctulata* spermatozoa by 40% (Fig. 7). *A. punctulata* jelly, in contrast, required only about 60 nmol/ml of fucose for an equivalent inhibitory effect on sperm respiration (not shown).

The stimulation of jelly-inhibited *L. pictus* sperm respiration by speract was dependent on the concentration of the peptide; half-maximal stimulation of respiration occurred at about 1 nM in the presence of 63 nmol fucose/ml *(Arbacia)* or 799 nmol fucose/ml *(Lytechinus)* (Fig. 8). The concentration of speract required to half-maximally stimulate respiration at pH 6.6 is about 50 pM (Fig. 8).

DISCUSSION

It was previously reported by Kinsey et al. (1979) that egg jelly of *S. purpuratus* initiates the acrosome reaction of homologous sperm cells with a resultant decrease in respiration rates, and recently Christen et al. (1983) have reported that jelly-treated *S. purpuratus* sperm cells alkalinize after the induction of an acrosome reaction followed by a reacidification and a resultant decrease in respiration rates. In both reports, jelly-treated spermatozoa appeared to lose viability rapidly. Here, we show that the jelly-inhibited respiration of sea urchin spermatozoa can be reversed by the subsequent addition of speract or of resact, two peptides which elevate sperm respiration rates in a species-specific manner. The respiratory inhibition by the egg jelly did not seem to be necessarily related to the induction of an acrosome reaction. The inhibitory component of jelly appeared to be nondialyzable, was active between pH values of 6.6–7.8, and was present in heterologous egg jelly which did not induce an acrosome reaction. However, the relief of jelly-inhibited respiration by speract was transient in the homologous system where acrosome reactions occur. Under acidic extracellular pH conditions, which have been used for the initial detection and isolation of speract, resact and similar peptides, stimulatory effects also often appear to be transient (Suzuki et al., 1983). Since the fertilizing capacity of sea urchin spermatozoa is reported to be decreased dramatically in less than 1 min after the induction of an acrosome reaction (Kinsey et al., 1979; Vacquier, 1979), the maintenance of motility and respiration for long periods of time is probably not necessary.

In both the heterologous and homologous system, monensin A which catalyzes electrically neutral Na^+/H^+ exchange across cell membranes, reproduced the effect of speract and of resact on sperm respiration, suggesting that jelly-inhibited respiration may be relieved by an elevation of intracellular pH. However, other agents which elevate sperm intracellular pH have yet to be tested.

These data suggest that sea urchin sperm cells normally become inhibited as they traverse the egg extracellular matrix (jelly) and that speract, resact and peptides from other species of sea urchins reverse the inhibition. The reversal would appear to be transient but of sufficient length in time for fertilization to occur (Vacquier, 1979). Since the peptides show some degree of species specificity, they could function to prevent cross-fertilization between various species. It now remains to be determined, however, whether or not fertilizaton rates can be altered by the manipulation of these peptides. It also remains to be determined whether or not the actual physiological function of the peptides is to stimulate sperm respiration and motility or to alter some other yet unmeasured physiological parameter.

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REFERENCES

Christen, R., Shackman, R. W. and Shapiro, B. M. (1983). Interactions between sperm and sea urchin egg jelly. Dev. Biol. 98:1–14.

Dan, J. C. (1952). Studies on the acrosome. I. Reaction to egg water and other stimuli. Biol. Bull. 103:54–66.

Dische, Z. and Shettles, L. B. (1951). A new spectrophotometric test for the detection of methylpentose. J. Biol. Chem. 192:579–582.

Garbers, D. L., Watkins, H. D., Hansbrough, J. R., Smith, A. and Misono, K. S. (1982). The amino acid sequence and chemical synthesis of speract and of speract analogues. J. Biol. Chem. 257: 2734–2737.

Goldstein, L., Levin, Y. and Katachalski, E. (1964). A water-insoluble polyionic derivative of trypsin. II. Effect of the polyelectrolyte carrier on the kinetic behavior of the bound trypsin. Biochemistry 3:1913–1919.

Hansbrough, J. R. and Garbers, D. L. (1981). Speract. Purification and characterization of a peptide associated with eggs that activates spermatozoa. J. Biol. Chem. 256:1447–1452.

Hathaway, R. R. (1963). Activation of respiration in sea urchin spermatozoa by egg water. Biol. Bull.

125:486–498.

Holland, L. Z. and Cross, N. L. (1983). The pH within the jelly coat of sea urchin eggs. Dev. Biol. 99:258–260.

Isaka, S., Hotta, K. and Kurokawa, M. (1970). Jelly coat substances of sea urchin eggs. I. Sperm isoagglutination and sialopolysaccharide in the jelly. Exp. Cell Res. 59:37–42.

Ishihara, K., Oguri, K. and Taniguchi, H. (1973). Isolation and characterization of fucose sulfate from jelly coat glycoproteins of sea urchin eggs. Biochim. Biophys. Acta 320:628–634.

Kinsey, W. H., SeGall, G. K. and Lennarz, W. J. (1979). The effect of acrosome reaction on the respiratory activity and fertilizing capacity of echinoid sperm. Dev. Biol. 71:49–59.

Kopf, G. S. and Garbers, D. L. (1980). Calcium and a fucose-sulfate-rich polymer regulate sperm cyclic nucleotide metabolism and the acrosome reaction. Biol. Reprod. 22:1118–1126.

Levin, Y., Pecht, M., Goldstein, L. and Katchalski, E. (1964). A water-insoluble polyanionic derivative of trypsin. I. Preparation and properties. Biochemistry 3:1905–1913.

Lillie, F. R. (1913). Studies on fertilization. V. The behavior of the spermatozoa of *Nereis* and *Arbacia* with special reference to egg extracts. J. Exp. Zool. 14:515–574.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

Nomura, K., Suzuki, N. and Isaka, S. (1983). Structure and activity of sperm activating peptides from the egg jelly of Japanese sea urchins. In Peptide Chemistry 1982. (S. Sakakibara, ed.). Protein Research Foundation, Osaka, pp. 171–176.

Ohtake, H. (1976). Respiratory behavior of sea-urchin spermatozoa. I. Effect of pH and egg water on the respiratory rate. J. Exp. Biol. 198:303–312.

Repaske, D. R. and Garbers, D. L. (1983). A hydrogen ion flux mediates stimulation of respiratory activity by speract in sea urchin spermatozoa. J. Biol. Chem. 258:6025–6029.

SeGall, G. K. and Lennarz, W. J. (1979). Chemical characterization of the component of the jelly coat from sea urchin eggs responsible for induction of the acrosome reaction. Dev. Biol. 71: 33–48.

Suzuki, N., Nomura, K., Ohtake, H. and Isaka, S. (1981). Purification and the primary structure of sperm-activating peptides from jelly coat of sea urchin eggs. Biochem. Biophys. Res. Commun. 99:1238–1244.

Suzuki, N., Ohizumi, Y., Yasumasu, I. and Isaka, S. (1983). Respiration of sea urchin spermatozoa in the presence of a synthetic jelly coat peptide and ionophores. Dev. Growth Differ. 25 (in press).

Umbreit, W. W. (1964). In Manometric Techniques, 4th ed. (W. W. Umbreit, R. H. Burris, and J. H. Stauffer, eds.). Burgess Publishing Co., Minneapolis, p. 5.

Vacquier, V. D. (1979). The fertilizing capacity of sea urchin sperm rapidly decreases after induction of the acrosome reaction. Dev. Growth Differ. 21:61–69.

Vasseur, E. and Hagstrom, B. (1946). On the gamones of some sea urchins from the Swedish west coast. Ark. Zool. 37A:1–17.