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INDUCTION OF POLYSPERMY IN SEA URCHIN EGGS BY ANTIBODIES RAISED AGAINST A HAMSTER SPERM PROTEIN

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Polyclonal antibodies raised against a hamster sperm protein (P26h) induce polyspermic fertilizations in the green sea urchin without affecting the fertilizing ability of the spermatozoa nor the elevation of the fertilization membrane. While the adsorption of the antibodies on sperm decreased the polyspermic effect, preincubation of unfertilized eggs with the anti-P26h did not cause polyspermy. These results suggest that common epitopes are involved in fertilization processes in phylogenetically distant species.

In sea urchins, gamete interaction is mediated by a sperm protein, bindin, and its receptor on the egg surface (1). The mammalian counterpart of this cell interaction process occurs when the spermatozoa reach the zona pellucida, an acellular coat surrounding the oocyte which delimits the perivitelline space. Whereas the mouse zona pellucida glycoprotein exhibiting sperm receptor properties has been well characterized, the identification of the sperm proteins involved in zona pellucida recognition remain controversial (2). Thus, the mammalian counterpart of the sea urchin bindin remains to be established.

Following spermatogenesis, mammalian spermatozoa transit into the epididymis. This organ secretes proteins that are involved in sperm maturation. This process is defined by the acquisition by the spermatozoa of their fertilizing ability, namely the zona pellucida binding ability (3). We have previously identified a hamster epididymal sperm protein (P26h) which is involved in the processes mediating the interactions between the spermatozoa and the zona pellucida (4,5). Indeed, a polyclonal antiserum raised against the P26h inhibits hamster sperm-zona pellucida interaction *in vitro* (5). In this study, we have investigated the effect of this antiserum on sea urchin gamete interactions. Surprisingly, rather than interfering with

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sperm-egg binding in this species, the anti-P26h antiserum induced polyspermy. These results are discussed with regard to a possible homology between proteins of invertebrate and mammalian spermatozoa that may be involved in different steps of gamete interaction.

MATERIALS AND METHODS

Anti-P26h antiserum. A rabbit polyclonal antiserum was raised against a cauda epididymal hamster sperm protein as described previously (5). Briefly, spermatozoa were recovered from the distal tubule of the cauda epididymis of mature hamsters, Mesocricetus auratus. After washing the sperm, membrane proteins were detergent extracted with a buffer containing 0,5% (v/v) Nonidet P-40. The extracted proteins were submitted to preparative SDS-PAGE according to (6) and the region of the electrophoretic pattern corresponding to the P26h was excised and used to immunize rabbits. The rabbit polyclonal antiserum has been shown to be specific for the epididymal and sperm P26h protein and to recognize only one polypeptide band on Western blots of two-dimensional electrophoretic patterns of hamster sperm proteins (5). The anti-P26h serum was decomplemented at 56°C and the IgGs were purified by affinity chromatography on Protein A-Sepharose (Pharmacia) according to the instructions of the supplier. After purification, the IgG fractions were dialysed against 0,5% (w/v) NH_4HCO_3, frozen, lyophilized, resuspended in artificial sea water (ASW) and extensively dialyzed against ASW. Protein determination was performed according to (7). IgGs from the preimmune serum, e.g. the serum obtained from the rabbits before immunization, were prepared according to the same procedure and used as a control.

Gamete handling and fertilization. The green sea urchins (Strongylocentrotus droëbachiensis) were induced to spawn by intracoelomic injection of 0.5M KCl. Eggs were recovered in ASW prepared according to (8) and the spermatozoa were kept "dry" at 4°C until use. Eggs were dejellied by filtration through cheesecloth and washed by gentle centrifugation. Egg suspensions were adjusted to a concentration of 1% (v/v) in ASW and fertilized at time zero with a predetermined dilution of sperm to obtain an optimal percentage of monospermic fertilizations. In some experiments, the sperm was further diluted in order to obtain a suboptimal percentage of fertilization. Experiments were conducted at 10°C as previously described (9).

Sea urchin eggs, at a concentration of 1% (v/v), were incubated in the presence of different concentrations of anti-P26h or preimmune IgGs. After 5 min. at 10°C, an optimal dilution of spermatozoa was added. Thirty min. later, development was stopped by fixing the eggs with a buffer containing 4% formaldehyde. The eggs were stained with the DNA-specific dye, Hoechst 33258, according to (10) and the percentages of fertilized or polyspermic eggs (e.g. two or more decondensing sperm pronuclei per egg) were determined with a minimum of 200 eggs observed under epifluorescence.

In some experiments, the IgG preparations were preadsorbed on spermatozoa. Solutions of 200 µg/ml of IgG in ASW were incubated on ice with different volumes of spermatozoa previously cleaned from "dry sperm" by centrifugation and pelleted. After 30 min, the spermatozoa were eliminated by centrifugation at 5 600 g on a cushion of 25% Percoll prepared in ASW. The supernatants were recovered and used to incubate the eggs 5 min. before fertilization.

RESULTS AND DISCUSSION

The fertilization rate of S. droëbachiensis as determined by counting spermatozoa that have penetrated the eggs or by the elevation of the fertilization membrane was similar whether the eggs were inseminated in ASW or in the presence of IgG from the preimmune serum or the anti-P26h. However, the anti-P26h induced polyspermic fertilizations in a dose-dependent manner (Fig. 1). At a concentration of 200 µg/ml of anti-P26h, the percentage of polyspermy was 82%. In comparison, fertilization achieved in the presence of the same concentration of preimmune IgG resulted in a percentage of polyspermy comparable to that

observed when no IgG was added to the ASW e.g. less than 5% (Fig. 1). When the kinetics of fertilization membrane elevation was monitored in ASW and in presence of anti-P26h or preimmune IgG, no difference was observed in the time course of the establishment of this slow block to polyspermy (data not shown). Therefore, the polyspermic property of the anti-P26h could not be explained by an effect on the slow and permanent block established by the cortical granule exocytosis. This was in agreement with the fact that the average number of decondensed sperm pronuclei in the eggs inseminated in presence of the anti-P26h was generally limited to three per egg. This relatively low number of penetrated spermatozoa suggests that the block to polyspermy was not completely abolished by the IgG anti-P26h. This polyspermic effect was rather unexpected considering that the anti-P26h antibodies exhibit an inhibitory effect on gamete interaction in the hamster (4,5).

In order to further document the polyspermic effect of the anti-P26h, solutions of 200 µg/ml of IgG were preadsorbed on different volumes of spermatozoa of S. droëbachiensis and then used to prepare 1% (v/v) suspensions of eggs that were inseminated with a monospermic concentration of spermatozoa. This adsorption significantly lowered the polyspermic effect of the anti-P26h IgG. The loss of the polyspermic property of the anti-P26h was proportional to the volume of sperm on which the antiserum had been previously adsorbed (Fig. 2). When the unfertilized eggs were preincubated with the anti-P26h IgGs, washed and then inseminated in the absence of soluble IgG, no increase in polyspermy was observed (data not shown). Thus, it appears that the polyspermic effect of the anti-P26h was mediated via a sperm component.

One way to explain the polyspermic effect of the anti-P26h would be an increase of the sperm fertilizing ability through an increased motility or a greater fusion capacity. If this were the case, one would expect a higher percentage of fertilized eggs, in the presence of the anti-P26h, at suboptimal sperm concentrations. When fertilization was performed in the presence of IgG using a suboptimal concentration of spermatozoa, the total percentage of fertilized eggs was similar whether the insemination was performed in ASW with or without preimmune or anti-P26h IgG (Fig. 3). The polyspermic effect of the anti-P26h, compared to the preimmune IgG, remained highly significant with a $P < 0.001$ (Fig. 3). This suggests that the anti-P26h interferes with the establishment of the fast block to polyspermy in sea urchin eggs without increasing the fertilizing ability of the spermatozoa.

In the sea urchin, gamete interaction is mediated by a protein-glycoprotein interaction, e.g. between the sperm bindin and its receptor on the egg surface. Indeed, insoluble bindin particles agglutinate unfertilized eggs in a species specific fashion (11). This binding does not induce egg activation (1). Thus, sperm components other than bindin may be involved in egg activation. In echinoderm species, the earliest detectable event occurring after the sperm makes contact with the egg is the formation of an aqueous pore between the two gametes (12). Thus, rapidly after gamete contact, the diffusion of cytosolic substances from the sperm to the eggs could occur as suggested by the reported ability of injected soluble sperm extracts to activate eggs (13). Similarly, in mammals, soluble cytosolic protein factors from the spermatozoa have been shown to induce egg activation (14). Mammalian sperm surface

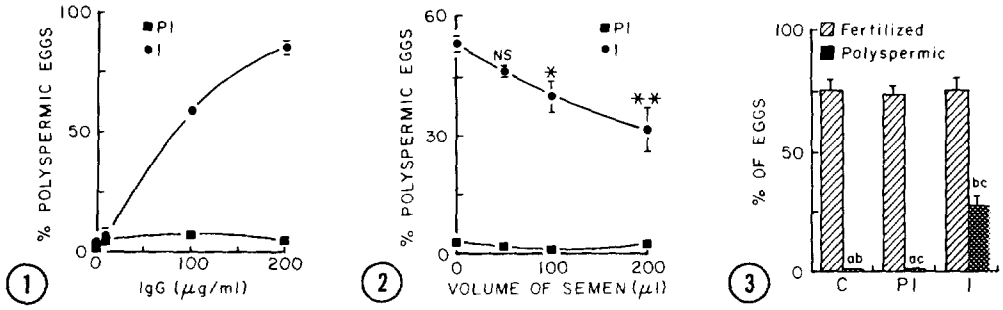

FIGURE 1. Induction of polyspermy by the anti-P26h. Oocytes (1% v/v), incubated for 5 min. in ASW alone or in presence of different concentrations (μg/ml) of preimmune (P) or anti-P26h (I) IgG, were inseminated with a monospermic concentration of spermatozoa. 30 min. later the eggs were fixed, stained with Hoechst 33258 and the percentages of polyspermy determined by counting 200 eggs. Mean results (±SEM) of three determinations.

FIGURE 2. Polyspermic effect of the anti-P26h preadsorbed on spermatozoa. A solution of 200 μg/ml of preimmune (PI) or anti-P26h (I) IgG was incubated for 30 min. on ice with different volumes of spermatozoa. After elimination of the sperm, the IgG solutions were used with 1% (v/v) egg suspensions. These eggs were inseminated with a monospermic concentration of sperm. Percentages of polyspermy were determined by counting 200 Hoechst 33258-stained eggs. Mean results (±SEM) of three determinations. Compared to the unadsorbed serum, adsorption of the anti-P26h on dry sperm significantly lowered the percentage of polyspermy with a $p < 0.01$ (*) or $p < 0.001$ (**). NS: not significant

FIGURE 3. Effect of the anti-P26h on suboptimal fertilization in sea urchin. Suspensions of 1% (v/v) eggs were incubated for 5 min. in ASW (C) or with 200 μg/ml of preimmune (PI) or anti-P26h (I) IgG and inseminated with a suboptimal concentration of sperm. Percentages of monospermic and polyspermic fertilizations were evaluated on 600 Hoechst 33258-stained eggs. Percentages of polyspermy were determined on fertilized eggs only. Mean results (±SEM) of three determinations. a) values were not significantly different, whereas b) and c) values were different with a significance of $p < 0.001$.

antigens, when incorporated into the egg plasma membrane after fertilization, may also play a role in the activation of the developmental program of the zygote, especially zygote cleavage (15). Our results show that a sperm component present on sea urchin spermatozoa share some common epitopes with a hamster sperm protein (Fig. 2). In the hamster, the P26h protein is involved in gamete interaction, especially at the level of the zona pellucida (5). In the sea urchin, the antibodies recognizing the sperm antigen do not interfere with the percentage of fertilized eggs (Fig. 3) nor with the kinetics of the fertilization membrane elevation. The antibodies would appear to interfere with one of the earliest responses of the eggs following sperm binding, e.g. the establishment of the electrically-mediated fast block to polyspermy (16). These early bioelectrical responses of eggs are known to be triggered by sperm components (17), and extracted sperm proteins or even derived synthetic peptides are able to induce these electrical responses, as well as egg activation, in the echiurian *Urechis* (18). Under our conditions, the electrical response of the sea urchin egg could thus be mediated by a sperm component that is recognized by the anti-P26h. Further characterization of the common epitopes shared by spermatozoa of organisms phylogenetically as distant as sea urchin and mammals, should provide new insights into the possible universal mechanisms involved in fertilization.

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