Abstract The acrosome reaction is a fundamental requirement for mammalian fertilization. Its exact molecular mechanisms and cellular elements are still poorly understood. We have detected an acrosomal sperm antigen, SAA-1, by monoclonal antibodies directed against SAA-1, that appears to be critically involved in the regulation of the acrosome reaction. SAA-1 is conserved within a broad range of mammalian species, emphasizing its important role in mammalian reproduction. Here we demonstrate that SAA-1 is immunohistochemically detectable in a nonmammalian vertebrate whose sperm do not possess an acrosome. In the swordtail, a live-bearing teleost with special reproductive tactics, we were able to demonstrate immunoreactivity of sperm heads of spermatids and mature sperm in the testis using monoclonal antibodies against SAA-1. Due to the cystic spermatogenesis with synchronous sperm maturation, immunoreactive maturational stages could be clearly identified. Stored immunoreactive sperm were also identified in spermathecal tissue of the female genital tract. Interestingly, immunoreactivity was furthermore detected in defined cells of the compounded endocrine organs pituitary and endocrine pancreas. All these different cell systems are involved in paracrine regulation and exhibit exocytotic properties. The possible nature of SAA-1 is discussed. Additionally, some new aspects of the morphologic composition of the swordtail pituitary are described.

Keywords Acrosome reaction · Testis · Pituitary · Endocrine pancreas · Immunohistochemistry · Sperm antigen · Xiphophorus helleri (Teleostei)

Introduction

In mammals, fertilization consists of a series of timed, receptor-regulated events. Upon contact with the oocyte vestments, sperm from most species must undergo the acrosome reaction (AR), an exocytic event required for the penetration of the zona pellucida (ZP) and also for the generation of a membrane segment capable of fusing with the oolemma. The molecular mechanisms responsible for the regulation of the AR are still poorly defined, although knowledge in this area is rapidly expanding (for review, see Benoff 1998; Fraser 1998).

The physiologic AR occurs at the surface of the ZP following binding to zona pellucida protein 3 (ZP3; Beil and Wassarman 1989). In vitro, the AR can be induced by other agents thought to be involved in priming for a successful AR in vivo, including progesterone (Osman et al. 1989) and prostaglandin E (Schaefer et al. 1998). The AR is calcium-dependent; calcium entry through specific ion channels apparently mediates acrosomal exocytosis. Exposure of capacitated mouse or bovine spermatozoa to solubilized ZP glycoproteins results in two waves of calcium influx into spermatozoa: a transient rise in intracellular calcium (Storey et al. 1992), followed by a sustained calcium elevation most probably due to activation of L-type voltage-dependent calcium channels (Florman et al. 1992). The definite nature of calcium entry mechanisms in the context of the AR awaits further clarification.

The mechanisms of fertilization in submammalian vertebrates are much less understood than in mammals. Differences in sperm morphology as well as in fertilization strategies have to be considered. Thus, the acrosome which envelopes almost the entire nucleus in mammalian sperm is much less distinct in other vertebrate species. Teleost sperm have no acrosome at all. Internal and external fertilization as well as differently constructed oocyte vestments within different vertebrate classes may be the reason for these differences (for review, see Blüm 1986).

We have described an acrosomal sperm antigen, SAA-1, intimately involved in the regulation of the
sperm AR. Monoclonal antibodies (mabs) against SAA-1 inhibit mouse fertilization in vitro and in vivo (Brucker et al. 1992a, 1992b). In human sperm, these mabs inhibit the progesterone- and prostaglandin E-induced AR and attenuate progesterone-induced calcium influx (Brucker et al. 1994, 2000). The biochemical characterization of SAA-1, a molecule of 220,000 Da, has been hampered due to a limited source of antigen and the high protease susceptibility of this large protein. Meanwhile, a thorough histological characterization of SAA-1 has been conducted on the light- and electron-microscopic level, and the occurrence of SAA-1 in other cells and tissues has been studied. Also, phylogenetic aspects of conservation of this important antigen within different species have been clarified. So far, within testicular sections of vertebrate species, SAA-1 immunoreactivity (ir) was only detected in acrosomal structures of spermatids and sperm from mammals. Furthermore, some nontesticular human tissues displayed SAA-1 ir (Brucker et al. 1995, 2000). Here we show specific SAA-1 ir in a teleost fish, Xiphophorus helleri, within gonadal and extragonadal endocrine tissues.

Materials and methods

Animals

Wild-type swordtails, X. helleri, of the inbred strain Helleri III were obtained from the Department of Genetics of the Justus Liebig University, Giessen, Germany. Before being killed, these live-bearing teleosts were kept for several weeks at 24°C water temperature by a light/dark cycle of 14/10 h in mixed groups in 100-l-volume tanks. They were fed with dried food (Tetramin, Bremen, Germany). A total of 15 animals of different age and sex were investigated: 5 juvenile, immature, about 15-week-old animals; 5 adult, mature females; and 5 adult, mature males. Sex and maturational state of the animals were examined by histological analysis of the gonads. Sexual maturity was defined as presence of either mature follicles or spermatozeugmata. The Principles of laboratory animal care (NIH publication No. 86–23, revised 1985) as well as the current version of the German Law on the Protection of Animals were followed.

Histology

Fishes were killed by an excessive amount (0.3%) of anesthetizing chlorobutanol (Merck, Darmstadt, Germany) in water. Adult animals were subsequently decapitated and gonads were removed, whereas the small juveniles were fixed whole.

Bodies of juveniles, gonads, and whole heads of adult fish were fixed in Bouin’s solution (Bouin 1897) for 24 h at room temperature (RT). Fixation was followed by decalcification of the bodies and heads in 5% acetic acid for 24 h. Subsequently, tissues were dehydrated in ethanol (70%, 96%) and 2-propanol and embedded in Paraplast (Lancer, St. Louis, Mo., USA). Thin-sections of 5–7 μm were cut and applied to gelatin-coated slides.

Histology and immunohistochemistry

For identification of defined stages of spermatogenesis and differentiation of individual cell types in the examined tissues, selected sections were stained using the trichrome method of Goldner (1938) in combination with Gomori’s method (Gomori 1950), called aldehydefuchsin-Goldner (AFG) staining. In addition, the staining method according to Berg (1953) was used in testes and ovaries to demonstrate the presence of mature spermatozoa. Glycoproteins and carbohydrates were stained using periodic Schiff’s reaction (PAS) in aqueous media (McManus 1948).

For immunohistochemical studies, five mouse mabs directed against sperm antigen SAA-1 were used as primary antibodies, as indicated in the Results section. The generation and initial characterization of first-generation mab AG7 and second-generation mabs 2A1, 4C7, 2H1, and 5B4 are described in earlier publications (Brucker et al. 1992a, 1994, 1995). Briefly, first-generation mab AG7 was derived from immunization of female mice with the 100,000 g octylglucoside (OCT) supernatant of human sperm and selected due to its fertilization-inhibiting properties in the mouse model. Second-generation mabs were derived from female mice immunized with immunoaffinity-purified SAA-1 and selected for reactivity against OCT-solubilized human sperm and the staining pattern of indirect immunofluorescence (IF) against human sperm. They were further characterized using competitive binding studies in vitro and in vivo bioassays. According to these analyses, the mabs recognize at least two different epitopes on the SAA-1 antigen. Antibodies were stored lyophilized. Reactivity and specificity of the mabs were restested after reconstitution in PBS using radioimmunoassay against the 100,000 g supernatant of OCT-solubilized human sperm, as well as indirect IF on human sperm.

In addition, two polyclonal antisera, a dilution of 1–3 µg/ml or unspecific isotype-matched control mab (MOPC-IgG1; Sigma, Munich, Germany) at a concentration of 10 µg/ml was applied for 18–24 h at 10°C. In the case of applying GH-antiserum, a dilution of 1:10,000 was used. For detection of specific staining, an avidin-biotin (ABC) method using an antimouse secondary antibody for SAA-1 and anti-rabbit secondary antibody for GH was applied according to the supplier’s instructions (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, Calif., USA). Staining was visualized by incubation in 0.0005% 3,3′-diaminobenzidine-tetrahydrochloride (DAB; Sigma, Munich, Germany) and 0.003% H2O2 for approximately 10 min. To increase contrast by metallic nickel precipitation, DAB was dissolved in 50 mM malein-TRIS-buffer, pH 6.9, with 0.1 M NaCl and Tween 80 (Sigma-Aldrich, Seelze, Germany). Sections were incubated in normal horse serum (SAA-1 mabs) or normal goat serum (GH antisera) for 30 min at RT to block unspecific binding. Subsequently, one of the anti-SAA-1 mabs at concentrations of 1–3 µg/ml or unspecific isotype-matched control mab (MOPC-IgG1; Sigma, Munich, Germany) at a concentration of 10 µg/ml was applied for 18–24 h at 10°C. In the case of applying GH-antisera, a dilution of 1:10,000 was used. For detection of specific staining, an avidin-biotin (ABC) method using an antimouse secondary antibody for SAA-1 and anti-rabbit secondary antibody for GH was applied according to the supplier’s instructions (Vectastain Elite ABC kit; Vector Laboratories, Burlingame, Calif., USA). Staining was visualized by incubation in 0.0005% 3,3′-diaminobenzidine-tetrahydrochloride (DAB; Sigma, Munich, Germany) and 0.003% H2O2 for approximately 10 min. To increase contrast by metallic nickel precipitation, DAB was dissolved in 50 mM malein-TRIS-buffer, pH 6.0, with 0.1 M ammonium-nickel-sulfate-6-hydrate. With the exception of the blocking step, every incubation was followed by two washes in wash buffer. Following staining, sections were dehydrated again and embedded in Eukitt (O. Kindler, Freiburg, Germany). Some of the ir-stained sections were counterstained according to Löffler (Burck 1982), with methylene blue.

Immunofluorescence staining

Deparaffinization and rehydration were carried out as above. Sections were then blocked for 1 h in goat serum (Vector Laboratories, Burlingame, Calif., USA) at a 1:50 dilution in TRIS-HCI 0.05 M pH 7.6, incubated with primary mab at 3–10 µg/ml for 18–24 h at 10°C, washed in TRIS-HCI with 0.15 M NaCl, and incubated with dichlorotriazinylaminofluorescein (DTAF)-labeled goat-anti-mouse IgG antibody (Dianova, Hamburg, Germany) at a 1:200 dilution in TRIS-HCI for 2 h at RT. Sections were rinsed in