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RADIOIMMUNOREACTIVITY AND RECEPTOR BINDING ACTIVITY OF SPECIFIC EPIDIDYMAL PROTEINS OF THE RAT AND OTHER SPECIES

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Radioimmunoassay (RIA) and radioreceptor assay (RRA) for the 32K Rat Epididymal Protein (REP) have been developed. Washed intact rat epididymal spermatozoa were used as receptors in the RRA. The effects of epididymal and seminal fluids of other species in these assays were studied. The results showed that there is species specificity in the immunoreactivity and receptor binding activity of sperm coating proteins.

The rat cauda epididymidis has been shown to contain a specific protein of MW 32 000 [1]. This protein is secreted by the caput epididymidis and binds to high affinity receptor sites on sperm membrane during epididymal transit [2]. Interaction of this protein with spermatozoa may play an important role in the fertility of the animal [3]. Antiserum against this 32K Rat Epididymal Protein (REP) has been raised by immunization of rabbits. The purified anti-32K REP IgG was shown not to be able to cross the blood-epididymis barrier [4].

The epididymides of other species have also been shown to secrete sperm coating proteins with MW ranging from 28 000 to 40 000 [5,6,7]. Furthermore, human semen contains a specific sperm coating protein of MW 38 000 which is probably of epididymal origin [8]. It is of interest to see whether these sperm coating proteins share some common immunological and receptor binding activities, and whether there is species specificity in the interaction of epididymal proteins with epididymal spermatozoa. For these purposes, radioimmunoassay (RIA) and radioreceptor assay (RRA) for the 32K REP have been developed and the effects of epididymal and seminal fluids of other species in these assays were studied.

MATERIALS AND METHODS

Collection of epididymal and seminal fluids

Epididymal fluids from the rat, guinea-pig, hamster and dog were collected by cannulation of the caudal epididymal ducts. After the contents have been flushed out with isotonic saline, spermatozoa were seperated from the epididymal plasmas by centrifugation at 10 000 x g for 15 min. The clear supernatants were stored at -20°C.

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The semen of rabbits was collected by means of an artificial vagina [9]. Human semen samples were obtained from men of proven fertility. After liquefaction of the semen, the seminal plasmas were seperated from spermatozoa by centrifugation at 10 000 x g for 15 min at 4°C. The clear plasmas were stored at -20°C.

Radioimmunoassay for 32K REP

The 32K REP was isolated from the rat caudal epididymal fluid and purified by a number of chromatographic steps [2]. Monospecific antiserum to the purified protein was raised in rabbits and purified as described previously [4]. The 32K REP was iodinated by the Lactoperoxidase method [10] to a specific activity of 23 µCi/µg protein. The iodinated protein was seperated from free ^{125}I by gel filtration on Sephadex G-25 column (20 x 1cm). The double antibody procedure was employed for RIA in a buffer consisting of 0.01 M sodium phosphate of pH 7.6 containing 0.15 M NaCl, 1% bovine serum albumin, 0.005 M EDTA and 0.01% NaN_3 at room temperature. Antiserum to 32K REP was diluted to bind 35-50% of the total radioactivity added (5 000 cpm). Sheep anti-rabbit gamma globulin was used to seperate the bound and the free radioactivity.

Radioreceptor assay

Spermatozoa collected from the rat cauda epididymidis were used as receptors for the 32K REP. They were seperated from the epididymal plasmas by centrifugation at 800 x g for 15 min, washed and incubated with labeled 32K REP (as tracer) by the method previously described [2]. The incubation medium contained 200 µl PBS, 100 µl labeled 32K REP (about 500 000 cpm), 2 x 10^7 spermatozoa and the appropiate amount of unlabeled protein, epididymal or seminal fluid proteins. Specific binding was estimated from the difference in the amount of radiolabel retained in the absence and presence of 100-fold molar excess unlabeled 32K REP [2]. In most cases specific binding accounted for about 80% of the total binding.

RESULTS

Competitive binding curves of RIA for the 32K REP are shown in Fig. 1. Binding of [^{125}I]32K REP to the antiserum was inhibited in a parallel manner

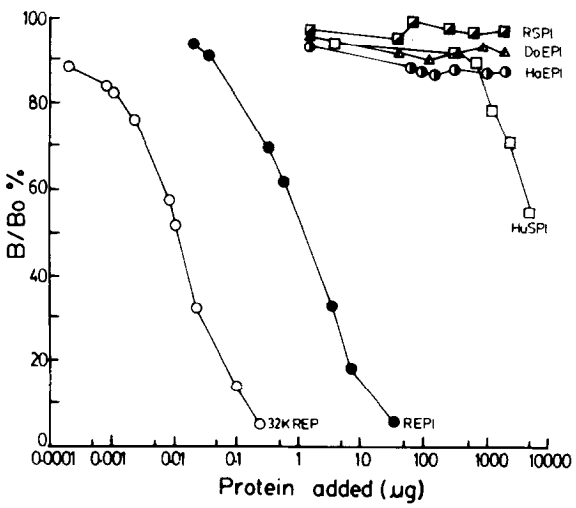

Figure 1: Displacement curves in the 32K REP RIA by unlabeled 32K REP, rat epididymal plasma (REP1), rabbit seminal plasma (RSP1), dog epididymal plasma (DoEP1), hamster epididymal plasma (HaEP1) and human seminal plasma (HuSP1). The standard (32K REP) and the REP1 curves are the mean from 5 and 4 assays respectively. The other curves are the mean of 2 assays.

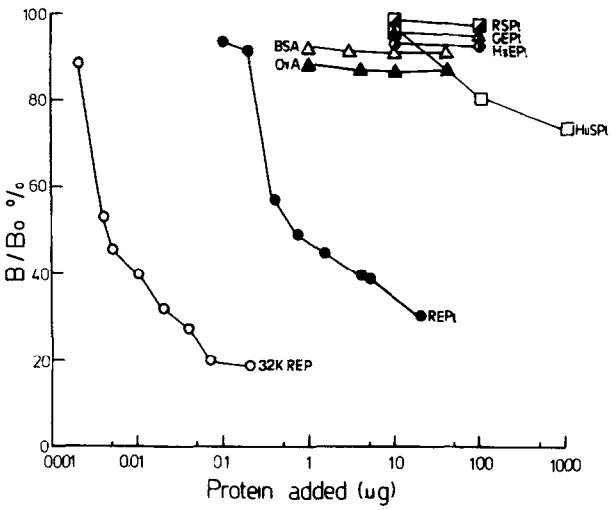

Figure 2: Displacement of the binding of [^{125}I]32K REP to washed intact rat epididymal sperm by unlabeled 32K REP, rat epididymal plasma (REP1), bovine serum albumin (BSA), ovalbumin (OvA), rabbit seminal plasma (RSP1), guinea-pig epididymal plasma (GEP1), hamster epididymal plasma (HaEP1) and human seminal plasma (HuSP1). The standard (32K REP) and the REP1 curves are the mean from 4 assays. The other curves are the mean from 2 assays.

by unlabeled 32K REP and rat epididymal plasma. The concentration required by the latter was about 100 times higher than the purified protein. No cross reaction was seen with rabbit seminal plasma, dog epididymal plasma and hamster epididymal plasma. The human seminal plasma gave < 0.01% cross reaction with the anti-rat epididymal protein IgG.

The displacement of binding in the 32K REP RRA by epididymal and seminal fluids of other species is similar to that in the 32K REP RIA (Fig. 2). The ID_{50} values (50% inhibition of binding) for the purified unlabeled 32K REP in the RIA and RRA were 10.4±1.7 ng (n=5) and 4.4±0.5 ng (n=4) respectively. The corresponding values for the rat epididymal plasma were 1.0±0.22 μg (n=4) and 0.59±0.03 μg (n=4) respectively. The human seminal plasma showed < 0.01% cross reaction in the 32K REP RRA. No cross reaction was observed with bovine serum albumin, ovalbumin, rabbit seminal plasma, guinea-pig epididymal plasma and hamster epididymal plasma (Fig. 2).

DISCUSSION

The results show that there is a good correlation in the inhibition of binding by the unlabeled 32K REP, rat epididymal plasma and human seminal plasma in both assays. The lack of cross reactivity of proteins from the reproductive tract fluids from other species in both assays would suggest that sperm coating proteins from other species are immunologically different to the 32K rat epididymal protein and that there is species specificity in the interaction of specific epididymal proteins with epididymal spermatozoa.

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