Vol. 100, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 29, 1981 Pages 840-846

LACK OF CYTOSOL AND NUCLEAR ESTROGEN RECEPTORS IN HUMAN SPERMATOZOA

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Received March 13, 1981

SUMMARY The presence of cytosol estrogen receptor in human spermatozoa was assayed using charcoal adsorption and hydroxylapatite procedures. With either of the two methods used, no specific binding of [^{3}H]-estradiol-17β to a macromolecular component was observed, indicating the absence of an estradiol receptor in human spermatozoal cytosol. Utilizing a [^{3}H]-estradiol nuclear exchange assay, no nuclear estrogen receptor could be detected.

INTRODUCTION

The presence of steroid hormones in both human semen and the female genital tract fluids is well documented (1-6). Human spermatozoa are able to interact with these steroids as they traverse the male and the female genital tracts. The levels of steroid hormones in the mammalian genital tract fluids in the female are significantly higher than their levels in plasma (6-8). Other studies have established that steroids can have effects on the motility, migration and/or metabolism of human spermatozoa *in vitro* and *in vivo* (9-15). It has been reported that estradiol-17β can increase human spermatozoal forward migration (9, 10), oxygen uptake (11, 12), lactate production (11, 12), pyruvate utilization (12), and [^{3}H]-tetracycline binding capacity (16) *in vitro*. However, the underlying mechanism(s) for these reported effects is unknown. The current general concept of the mechanism of steroid hormone action (17-19) is that the steroid hormone enters the target cell by diffusion and, in addition, perhaps by active processes (19). It is then bound to high affinity cytosol receptors that translocate it to the

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0006-291X/81/100840-07$01.00/0

nucleus, where interaction with the genetic material of the cell gives rise to synthesis of new proteins. This process involving gene action, takes much longer than the response of mammalian spermatozoal motility to steroid treatment, which is very rapid (9, 10, 20). Moreover, it has been reported that the binding of steroids, and estradiol-17β in particular, is highest in the mid-piece region, followed by the head, and then the tail (21). If the mechanism whereby a steroid influences spermatozoal activities involves interaction of the steroid with the genetic material in the nucleus, it would be expected that the majority of steroid would bind to the head region. In view of the above observations it seems that the molecular mechanism of steroid hormone action in human spermatozoa may differ from that commonly operating in other body cells. Since the specific binding of estradiol-17β to washed, but otherwise intact, human spermatozoa has been well documented (16, 21-24), it was decided to undertake a study to assay the cytosol and nuclear estrogen receptors in human spermatozoa in order to probe the molecular mechanism of steroid hormone action in spermatozoa.

MATERIALS AND METHODS

Chemicals. [2,4,6,7(n)-^{3}H]-estradiol-17β (104 Ci/mmole) was obtained from the Radiochemical Centre, Amersham, England. Hydroxylapatite and diethylstilbestrol were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Buffers. TESH buffer contained 10 mM Tris, 1.5 mM Na_4EDTA, 1 mM dithiothreitol, and was adjusted to pH 7.4 with 0.1 M HCl. TE buffer contained 10 mM Tris, 1.5 mM Na_4EDTA adjusted to pH 7.4 with 0.1 M HCl. Dextran-coated charcoal contained 0.05% dextran (T-70, Pharmacia, Sweden) and 0.2% charcoal (Fisher Scientific Company, NJ, USA) in TESH buffer. Hydroxylapatite suspension was prepared by washing hydroxylapatite three times with TE buffer and resuspending to a final concentration of 60% hydroxylapatite.

Human semen. Samples of human semen from normal healthy donors were collected by masturbation into clean glass jars after a minimum of 48 hr sexual abstinence. Samples were liquefied at 37°C and pooled for use within 30 min after collection. Sperm concentrations varied between 50 and 120 x 10^6 sperm/ml, and only those samples with motility and normal morphology greater than 80% were used.

Preparation of sperm cytosol. Pooled semen samples were washed twice with Krebs-Ringer-phosphate buffer (Ca^{2+}-free) (KRP)(25) at room temperature. After the final wash, the sperm concentration was adjusted to 1 x 10^8 sperm/ml with ice cold TESH. The samples were then homogenized with a motor-driven glass-

Teflon homogenizer for 45 sec/ml at 4°C. The homogenate was then sonicated at 4°C for 60 sec/ml with a Sonipen (Technic International Inc. USA) (settings used were: power 8, tune 4). The sonicated homogenate was centrifuged at 48,000 g for 20 min. The supernatant fraction was used as the sperm cytosol source.

Preparation of sperm nuclei. Sperm nuclei were isolated from human spermatozoa by the procedure of Marushige and Marushige (26), which involves homogenization, detergent treatment and centrifugation, with minor modifications. Ten grams of pooled semen samples was diluted to 35 ml with KRP and washed twice in this buffer by centrifugation at 1500 g for 10 min. The washed sperm pellet was resuspended in 15 ml of 10 mM Tris-HCl (pH 8) and homogenized with a motor-driven glass-Teflon homogenizer for 45 sec/ml at 4°C. The homogenate was then sonicated at 4°C for 60 sec/ml with a Sonipen (settings used were: power 8, tune 2). The resulting suspension was layered on an equal volume of 0.6 M sucrose-10 mM Tris-HCl (pH 8) and processed according to the procedure previously described (26).

Identification of cytosol estrogen receptor. Each assay consisted of triplicate samples. Aliquots of the cytosol fractions (500 µl) were added to two parallel series of tubes; one series contained the [^{3}H]-steroid with concentrations ranged between 0.1 - 10 nM, and the other contained the same concentrations of [^{3}H]-steroid as in the first series plus a 100-fold excess non-radioactive diethylstilbestrol. Cytosol fractions were incubated at 30°C for between 60-95 min. After this incubation period, the bound and free [^{3}H]-steroids were separated by either a charcoal adsorption or a hydroxylapatite procedure based on that of Clark and Peck (19).

Identification of nuclear estrogen receptor. Aliquots of the nulcear fraction (250 µl) were added to two series of tubes. To one series, [^{3}H]-steroid was added to a final concentration of 0.1 - 10 nM; the second series contained the same concentration of [^{3}H]-steroid plus a 100-fold excess non-radioactive diethylstilbestrol. The assay tubes were incubated for 60 min at 37°C to ensure 90-95% maximal binding (19). After this incubation period, the bound and free [^{3}H]-steroids were separated by the procedure previously described (19).

Other methods. Specifically bound [^{3}H]-estradiol-17β (i.e. that bound to estradiol receptor) was estimated by the subtraction of nonspecifically bound [^{3}H]-estradiol-17β (i.e. the amount of [^{3}H]-estradiol-17β bound in the presence of a 100-fold excess of unlabeled diethylstilbestrol) from total [^{3}H]-estradiol-17β binding (i.e. [^{3}H]-estradiol-17β bound in the absence of unlabeled diethylstilbestrol). The presence of steroid receptor was analysed by saturation analysis. Radioactivity was measured with a Packard 3255 liquid scintillation spectrometer. Insta-gel purchased from Packard was used as the scintillation fluid. All samples were examined for quench by automatic external standardization. The efficiency of the tritium measurement was 28-40%. Total protein was determined by the method of Lowry et al. (27) using bovine serum albumin as a standard. DNA concentration was measured by the method of Burton (28) using calf thymus DNA as a standard.

RESULTS

With the incubation procedure used to measure the total estradiol binding sites (oocupied and unoccupied) in sperm cytosol, based on that of Clark and Peck (19), no specific binding of [^{3}H]-estradiol to a macromolecular component could be detected when either the charcoal adsorption (Fig. 1a) or

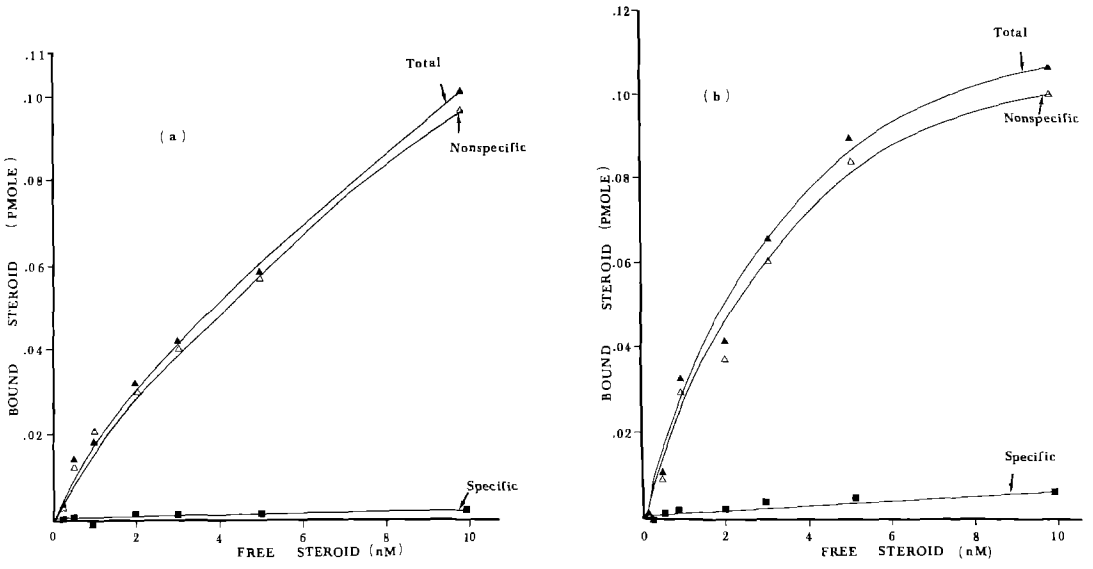

Fig. 1 a,b. Saturation analysis of [^{3}H]-estradiol-17β binding to sperm cytosol using either (a) the charcoal adsorption procedure (b) or the hydroxylapatite procedure to separate bound from unbound [^{3}H]-estradiol-17β. The cytosol (500 µl; 0.06 mg protein/ml) was incubated with [^{3}H]-estradiol-17β (0.1 - 10 nM) with or without a 100-fold excess of unlabeled diethylstilbestrol for 60-95 min at 30°C. Specific binding (■) is determined by subtracting non-specific binding (△) from total (▲). All points are the means of 6 determinations from 2 separate experiments.

the hydroxylapatite procedure (Fig. 1b) was used to separate the bound from the unbound [^{3}H]-estradiol. Also, no specific binding of [^{3}H]-estradiol to a nuclear estrogen receptor was identified (Fig. 2). In all instances, the nonspecific binding of [^{3}H]-estradiol by either cytosol or nuclear fraction in each assay tube represented only about 1% of the total counts added.

DISCUSSION

The present investigation indicates that both cytosol and nuclear estrogen receptors are absent in human spermatozoa. This provides further evidence that the molecular mechanism of steroid hormone in human spermatozoa may differ from that commonly operating in other body cells (17-19). The principal role of the spermatozoon is to carry the genetic material in its head, traverse the female genital tract and fuse with the egg so as to

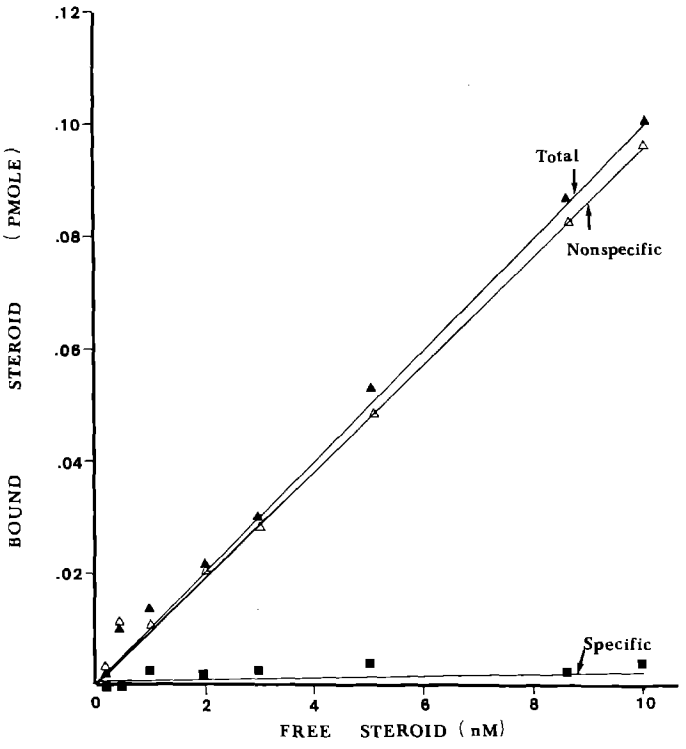

Fig. 2. Saturation analysis of [^{3}H]-estradiol-17β binding to sperm nuclear fraction. The nuclear fraction (250 µl; 20 µg DNA/ml) was incubated with [^{3}H]-estradiol-17β (0.1 - 10 nM) with or without a 100-fold excess of unlabeled diethylstilbestrol for 60 min at 37°C. Specific binding (■) is determined by subtracting nonspecific binding (△) from total (▲). All points are the means of 6 determinations from 2 separate experiments.

restore the chromosome number which is needed for subsequent embryonic development. It has been concluded that the genes in spermatozoa are inactive (29). There are reports which indicate that steroid hormones can exert their effects at the plasma membrane either by inducing alteration in membrane lipids which subsequently alters the rate of ion transport through the plasma membrane in toad bladder, rat liver and brush border membranes (30), or by altering the intracellular Ca^{2+} distribution which subsequently induces or inhibits meiotic division in Xenopus laevis oocytes (31-33). However, whether steroids exert their effects on spermatozoa through similar mechanisms is not known. Work is now in progress to characterize the steroid receptors

in plasma membrane in spermatozoa, and to study their role in regulating sperm functions.

ACKNOWLEDGMENTS

We thank Dr. H.R. Tinneberg for stimulating discussions throughout this work. This work was supported by grants from the Ford Foundation (Grant no. 760-0528) and World Health Organization (Grant no. 74250).

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