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HUMAN SERTOLI CELLS *IN VITRO*: MORPHOLOGICAL FEATURES AND ANDROGEN-BINDING PROTEIN SECRETION

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Summary—Sertoli cells play a pivotal role in the regulation of spermatogenesis as they provide the anatomical basis of the blood–testis barrier In the present paper we report some results of our studies on the ultrastructural features, the responsiveness to FSH, and the ability to secrete androgen-binding protein (ABP) of human Sertoli cells *in vitro* The nucleus showed the characteristic foldings of the nuclear membrane, scattered chromatin, and a fibrillar nucleolus In the cytoplasm Charcot–Boettcher crystals were present and active phagocytic activity was documented by the presence of vacuoles containing lipids and cellular debris Human Sertoli cells in culture responded to FSH with a maximal rise in cAMP that was approx 3-fold This response to FSH is comparable to that reported for the adult rat but lower than that of the immature rat, and suggests that human as well as rat Sertoli cells could have a reduced response to FSH since sexual maturation was achieved As no evidence has been reported on ABP secretion by human Sertoli cells in culture we evaluated the concentration of this protein in the Sertoli cell spent media Human Sertoli cells in culture produced ABP and the response to FSH was dose-related The K_d value of human ABP (hABP) was approx 7 5 nM, being slightly higher than that of the rat ABP and an order of magnitude different from that of sex hormone-binding globulin (SHBG) present in human plasma We also measured the association and dissociation rates of dihydrotestosterone–hABP complexes and the K_d/K_a ratio was very close to the value of K_d of the Scatchard analysis The differences between hABP and SHBG may open the way to the selective measurement of ABP in many conditions of male infertility

INTRODUCTION

Sertoli cells play a pivotal role in the regulation of spermatogenesis They provide the anatomical basis of the blood–testis barrier which segregates meiotic and postmeiotic germ cells Thus the microenvironment that makes it possible for the meiosis and spermiogenesis to take place is deeply conditioned by Sertoli cells [1, 2]

In vitro animal models have been especially helpful in the investigation of Sertoli cell function, but not much is known about human Sertoli cells [3–5] even though more information is badly needed for the understanding of the wide area of male infertility due to altered spermatogenesis, whose causes are still obscure In the present paper we report some results of our studies on the ultrastructural features, the responsiveness to FSH, and the ability to secrete androgen-binding protein (ABP) of human Sertoli cells *in vitro* ABP differs from sex hormone-binding globulin (SHBG) in its affinity for androgens and binding kinetics

MORPHOLOGICAL FEATURES OF HUMAN SERTOLI CELLS *IN VITRO*

The Sertoli cells spread into a continuous monolayer 24–36 h after seeding. At the electron microscope the nucleus showed an ovoidal shape with the typical enfoldment of the nuclear membrane and the chromatine was finely scattered (Fig 1) The nucleolus was composed of a fibrillar and compact portion, and only occasionally showed the complex tripartite structure with two cariosomes typical of rodent Sertoli cells [6]

The cytoplasm contained Charcot–Boettcher crystals, found only in human Sertoli cells, and microfibres and intermediate fibres which are constituted by vimentin in the rat [7] and have

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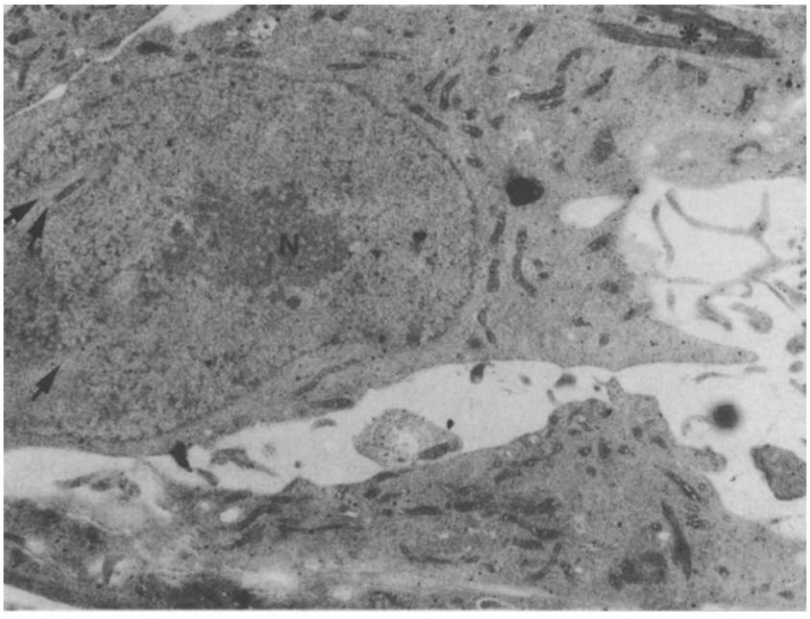

Fig 1 Ultrastructural features of human Sertoli cells in culture The ovoid nucleus shows foldings of the nuclear membrane (arrows) N nucleolus which displays a fibrillar aspect Charcot–Boettcher crystals (asterisk)

an important role in the structural changes of the cytoskeleton and thus of the Sertoli cell shape The Golgi complex, and the endoplasmic reticulum, both rough and smooth, were usually well developed Sometimes the smooth endoplasmic reticulum was organized in concentric "lamellae" surrounding lipid droplets and displaying pores ("annulatae lamellae") [8] The mitochondria had a double morphological pattern more frequently they were elongated and displayed tubular cristae, sometimes had a roundish shape

Vacuoles containing lipids (Fig 2), cellular debris and sometimes whole germ cells were frequently encountered suggesting that cultured Sertoli cells retained, or even enhanced, their phagocytic activity This activity was also documented indirectly by the histochemical positiv-

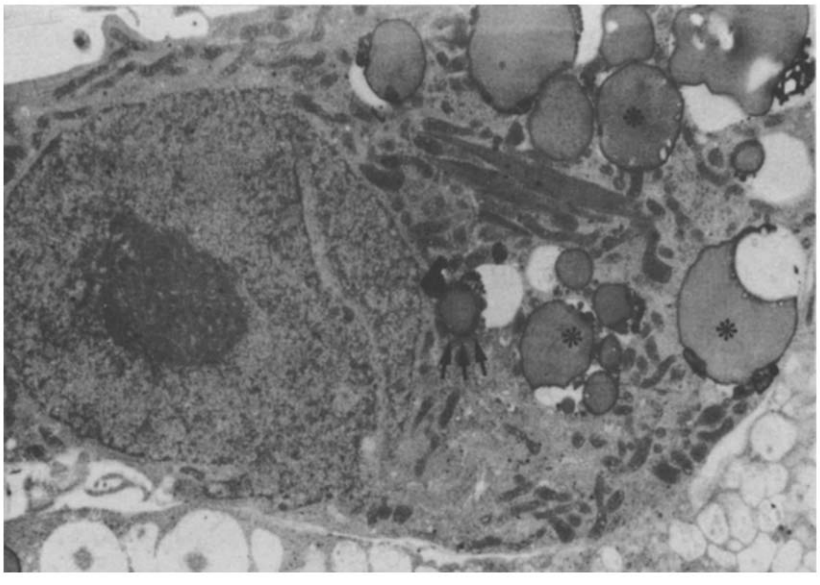

Fig 2 Ultrastructural aspects of phagocytic activity of human Sertoli cells in culture Membrane-bound lipid material (asterisk), and several vacuoles containing amorphous material are present (arrows)

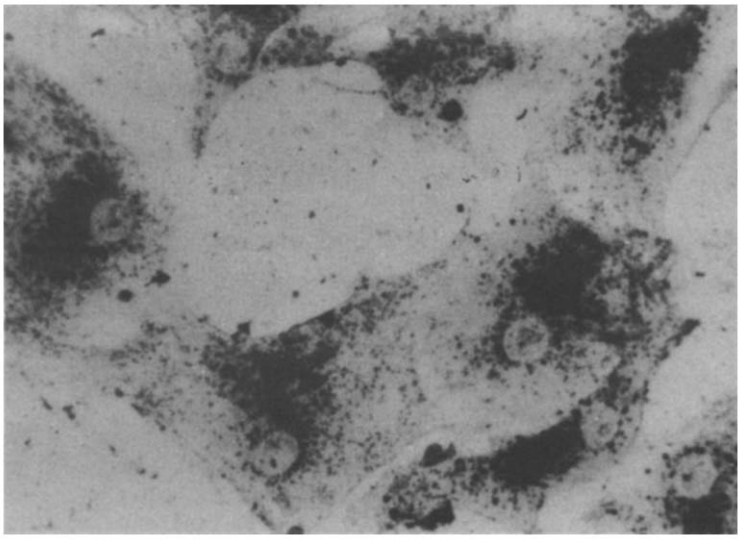

Fig 3 Marked histochemical positivity for acid phosphatase activity of human Sertoli cells *in vitro*

ity for acid phosphatase (Fig 3), as this enzyme is prevalently located in the lysosomes

In rat Sertoli cells both the number of the lysosomes and acid phosphatase activity are at their highest point at stage VII and VIII [9], during which spermiation and detachment of the "residual bodies" take place Phagocytosis of the residual bodies by Sertoli cells appears to be an autonomous activity not influenced by any hormonal stimulus [10], and has been proposed as a mechanism of local regulation of the spermatogenesis

cAMP RESPONSE TO FSH OF HUMAN SERTOLI CELLS *IN VITRO*

As many investigations, mainly on rodent Sertoli cells, have documented [1, 11], FSH stimulates many activities of these cells, including cellular duplication, changes in the cytoskeleton, and energy metabolism [12]

In the rat, the pattern of cAMP response to FSH is age-dependent the response progressively increases up to 18 days after birth, when it is maximal, and then abruptly decreases to remain blunted in adult animals [13, 14]

In our investigations adult human Sertoli cells in culture responded to FSH with a rise in cAMP, confirming that human, like other mammalian, Sertoli cells are responsive to FSH (Fig 4) The maximal response consisted of an approx 300% increase in cAMP, with an order of magnitude comparable to that reported for the adult rat, and definitely lower than that of the immature rat

Experimental evidence on the pattern of cAMP response to FSH with age in man is not available, but our data suggest that human as well as rat Sertoli cells could have a reduced response to FSH since sexual maturation was achieved

The cause of this phenomenon is not yet completely elucidated It has been suggested that in the adult rat the blunting of the response is modulated by the appearance of germ cells more mature than spermatogonia, e g spermatocytes and spermatids [15] Many functions of Sertoli cells, including the binding of FSH and ABP secretion, change along the stages of spermatogenesis [16] Also the cAMP response to FSH changes in a cyclic fashion and is maximal at stages I–VI

At the present time it cannot be excluded, but remains to be proven, that the reduced responsiveness to FSH might be due to, or have partially common mechanisms with,

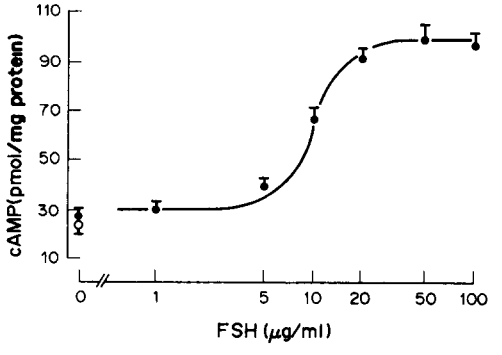

Fig 4 Dose-related cAMP response to FSH of human Sertoli cells in culture

desensitization, in which reduced adenylate cyclase activity and the activation of a phosphodiesterase with high affinity for cAMP were involved [17, 18]

ABP SECRETION BY HUMAN SERTOLI CELLS *IN VITRO*

ABP was first detected in the rat epididymis [19] and subsequently it was shown to be produced by Sertoli cells [20, 21] and considered a parameter of testicular function

The rat ABP consists of two subunits (41,000 and 45,000 Da on SDS–PAGE [22]) designated as rABP L and H [23] The two components are not present in a 1 1 ratio but rABP H and L occur in the ratio of 3 1 suggesting that the native rABP is not a simple heterodimer, but is a mixed hybrid system including the combinations of 45–45, 45–41 and 41–41 kDa dimers, in which the first two kinds of dimer predominate [23, 24]

The human ABP (hABP) in extracts of human testes is composed of two molecular species, based on concanavalin A (ConA)-Sepharose chromatography Form I hABP does not interact with ConA while Form II hABP binds to ConA [25]

The H and L protomers of Form I hABP were reported to have an apparent M_W of 55,000 and 52,000, and to usually be present in a 4 5 ratio (H L) The two components of Form II hABP have an apparent M_W of 53,000 and 48,000, respectively, and exist in a ratio of approx 20 1 [25]

SHBG, which is similar to Form II hABP with respect to ConA binding, has discrete H and L protomers in a 10 1 ratio Form I hABP differs from SHBG in ConA binding, carbohydrate structure, and perhaps in aminoacid sequence, as suggested by the different proteolytic patterns [25]

Comparison of the aminoacid sequences of rat ABP and hSHBG have shown that the two proteins each contain 373 residues and share 68% homology [26] This high degree of similarity existing between proteins from two different species suggests that they may be encoded by a single gene In addition, a comparison of the organization of the hSHBG and rat ABP genes indicates that they are well conserved and differ essentially only with respect to the sizes and sequences of introns between exons 5, 6, 7 and 8 [27] In conclusion, at the present time a largely shared opinion is that the difference in physicochemical properties between SHBG and ABP may be attributed to heterogeneity in carbohydrate composition

ABP is secreted bidirectionally into the seminiferous tubules down to the epididymis [28, 29] and, at least from days 15 to 40 of postnatal life in the rat, into the blood [30, 31] Testicular production in the rat is increased by FSH and testosterone [32] The rat ABP cDNA has been used to assess the influence of testosterone, FSH, and a combination of both hormones, on the relative amount of ABP mRNA in the testis of hypophysectomized animals *in vivo* [33], as well as in Sertoli cells grown *in vitro* [26] FSH increased the abundance of ABP mRNA, but testosterone increased ABP mRNA levels and augmented the effect of FSH only in the intact testis The inability of testosterone to induce ABP mRNA levels in cultured Sertoli cells supports the proposal that androgens may influence Sertoli cells indirectly by inducing a peritubular cell protein (PMod-S) that modulates Sertoli cell function [34]

As no evidence has been reported on ABP secretion by human Sertoli cells in culture, we evaluated the presence of ABP in the human Sertoli cell spent media by a specific binding assay on DEAE-Biogel In addition the effect of FSH on hABP secretion was assessed in cultures grown in chemically defined medium

Our data, elaborated according to the Scatchard plot analysis, documented that human Sertoli cells in culture produce ABP (28 4 ± 6 3 fmol/μg DNA/day) and that the response to FSH is dose-dependent, with a maximal production of ABP of 106 4 ± 20 8 fmol/μg DNA/day (Fig 5)

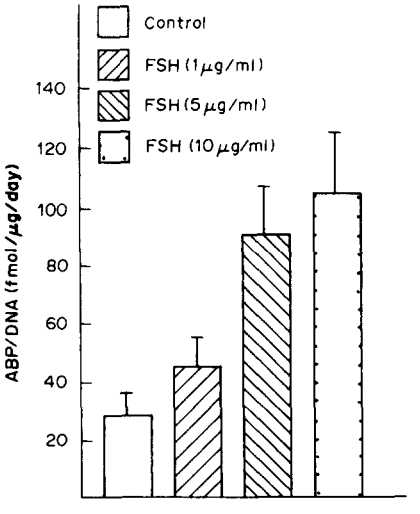

Fig 5 In human Sertoli cell cultures hABP secretion is stimulated in a dose-related fashion by FSH

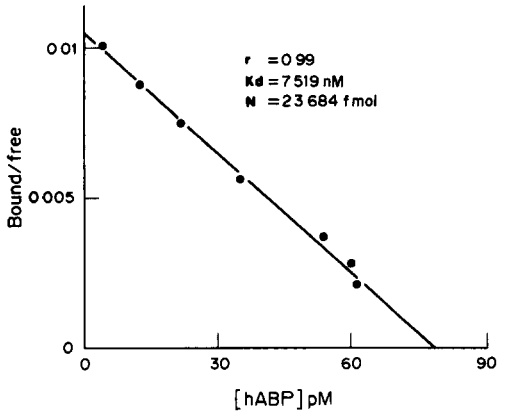

Fig 6 Scatchard analysis of hABP secreted by human Sertoli cells in culture

All values gave an equilibrium dissociation constant (K_d) approx 7 5 nM (Fig 6), corresponding to a high-affinity complex whose $K_a = 1\,4 \times 10^8\ M^{-1}$ The administration of FSH did not change the affinity of hABP for [^{3}H]dihydrotestosterone (DHT)

The association and dissociation rates of [^{3}H]DHT–hABP complexes were also evaluated For the measurement of the rate of association, 100 μl of media were incubated with [^{3}H]DHT for 3–180 min and the binding was evaluated by the Bio-Gel assay Figure 7(a) shows that the linear plot was consistent with a second-order reaction and that the slope corresponded to the association rate $K_a = 1\,15 \times 10^4\ M^{-1}\ S^{-1}$

The measurement of the dissociation rate of hABP–DHT complexes was performed after

Table 1 Some physicochemical and binding features of hABP, rABP and SHBG

	hABP	rABP	SHBG
K_d	7 5 nM	4 nM	0 7 nM
$t_{1/2}$ of complex (min)	100–140	3–6	70
Heat sensitivity	Sensitive at 50°C	Stable at 50°C	Sensitive at 50°C
Optimal pH for binding	8 (6–9)		8 (6 5–9)
Sialic acid content	+		–

exposition to a large excess of radioinert DHT for 3–360 min Figure 7(b) shows the reaction was of first-order and the dissociation rate constant was $K_d = 8\,4 \times 10^{-5}\ S^{-1}$ The half-life ($t_{1/2}$) of the hABP–DHT complexes was 137 5 ± 22 8 min The ratio of both dissociation and association rate constants (K_d/K_a) was 7 304, very close to the value of K_d obtained by Scatchard analysis

Our results show that the K_d value of hABP is slightly higher than that of the rat ABP [35, 36], an order of magnitude different from that [37] of SHBG present in human plasma

In Table 1 some differences among hABP, rat ABP and TeBG, based on the present and other reports, are listed Of particular interest are the differences between hABP and SHBG that may open the way to the selective measurement of hABP, as a marker of Sertoli cell function, in many conditions of male infertility

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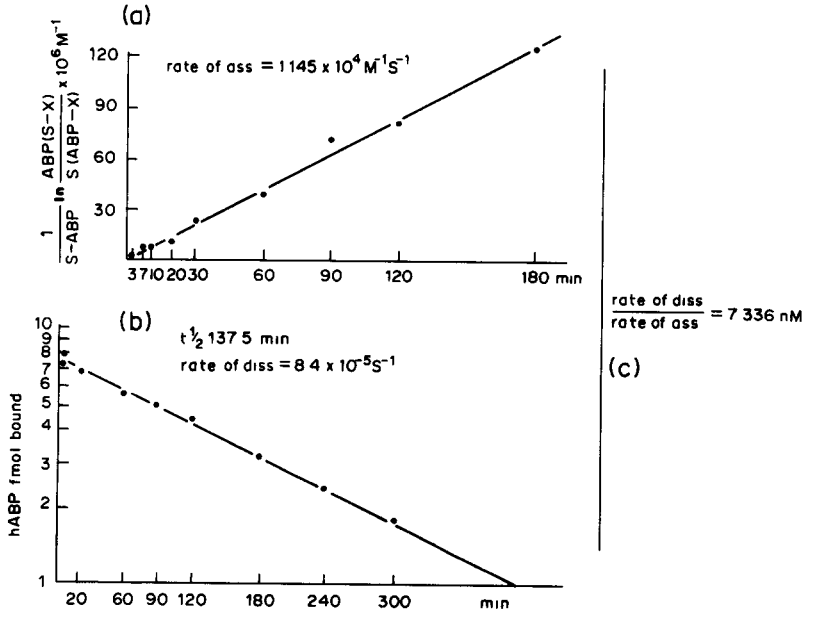

Fig 7 Kinetics of dihydrotestosterone-binding by hABP (a) Rate of association, and (b) rate of dissociation

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