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SERTOLI–GERM CELLS INTERACTIONS IN THE HUMAN TESTIS

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Summary—In previous histoimmunochemical studies we reported that transferrin (TF) and insulin-like growth factor I (IGF-I) are present in the cytoplasm of the Sertoli cells of the adult human testis Receptors for TF were found mainly in adluminal germ cells and type I receptors for IGF-I both in Sertoli and germ cells Using electron microscopy, evidence of transfer of both TF and IGF-I from the Sertoli to the germ cells through a receptor-mediated endocytosis mechanism was also found In this paper we report the results of the histoimmunochemical localization of α inhibin in the human fetal, prepubertal and adult testis In 8- to 14-week-old fetal testes a positive immunostaining was found mainly in the interstitial cells, whereas no staining was found in the germ cords In the prepubertal testis the immunostaining was present in the Sertoli cells but not in the interstitial cells In the adult human testis the immunostaining was present not only in the Sertoli cells but also in the spermatocytes and in several Leydig cells Using electron microscopy and immunogold labeling the presence of α inhibin immunoreactivity was found in the rough endoplasmic reticulum and in the Golgi cisternae of both Sertoli and Leydig cells Moreover we found evidence of transfer of α inhibin from the Sertoli to the germ cells through receptor-mediated endocytosis

INTRODUCTION

Several experimental data suggest that, to provide an appropriate milieu to the developing germ cells, the Sertoli cells secrete many proteins with different actions, such as transport proteins, growth factors, enzymes and basement membrane components [1] Most of the experimental data concerning Sertoli cell proteins and their relationships with germ cells have been obtained in experimental animals, whereas very few studies are available in man for obvious ethical reasons and because of the difficulties associated with the isolation of pure cell populations from human testicular tissue To overcome these difficulties we used histoimmunochemical techniques to study, in bioptic specimens of patients with obstructive azoospermia and normal spermatogenesis, some Sertoli cell proteins both with light and electron microscopy The results of our previous studies suggested that (1) transferrin (TF) and insulin-like growth factor I (IGF-I) are mainly localized in the cytoplasm of Sertoli cells [2, 3], (2) TF receptors are present mainly in adluminal germ cells [2], (3) type I IGF-I receptors are present both in Sertoli and germ cells [3], (4) TF and IGF-I are internalized into the basal compartment of the Sertoli cells through a receptor-mediated endocytosis mechanism [4], and (5) TF and IGF-I are transferred from the Sertoli cells into the germ cells through a receptor-mediated endocytosis mechanism [5]

In this paper we report the results of the histoimmunochemical localization of the α subunit of inhibin in the human testis Inhibin, a glycoprotein hormone that suppresses pituitary FSH, is a heterodimer of two partially homologous subunits (α-βA or α-βB) Immature and adult rat Sertoli cells in culture produce inhibin [5] and the mRNAs for α and β inhibin subunits have been recently demonstrated in these cells [6, 7] Recently the secretion of immunoreactive and bioactive inhibin was demonstrated by purified rat Leydig cells in culture and the mRNA for the α inhibin subunit was also shown in these cells [8] The possibility that Leydig cells have the capacity to secrete inhibin is also suggested by the increased levels of immunoreactive inhibin observed after LH or hCG administration both in humans [9] and rats [10] So it seems likely that both Sertoli and Leydig cells are involved in inhibin secretion

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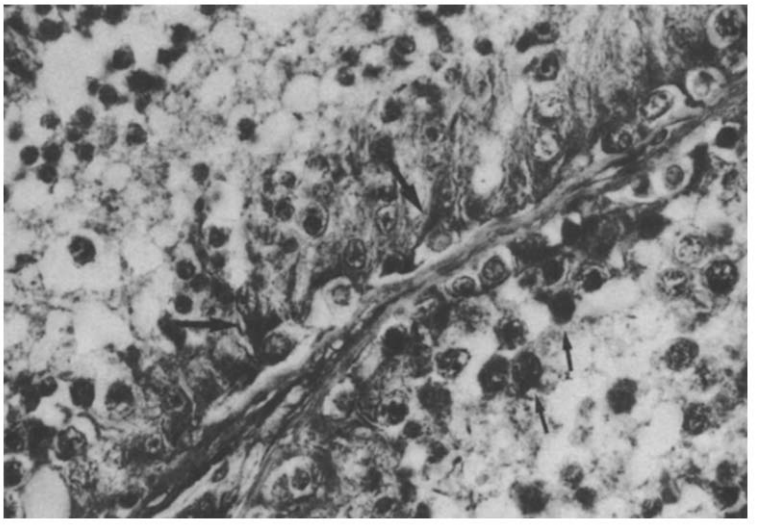

Fig 1 Immunostaining of the α inhibin subunit in the normal adult human testis The immunoreactivity is present both in Sertoli cells (large arrows) and in spermatocytes (small arrows) Magnification 385 ×

EXPERIMENTAL

The histoimmunochemical localization of α inhibin in the human testis was performed with an antiserum serum against the (1-26)-Gly-Tyr sequence of porcine α subunit (which was a kind gift of Dr W Vale) and with an antiserum against the 1-32-N terminal sequence of the human α subunit (which was provided by Dr A Negro-Vilar) The second antisera were peroxidase conjugates for light microscopy studies and coated with colloidal gold particles (15 nM dia) for electron microscopy studies Histoimmunochemistry was performed (1) in human fetal testicular tissue obtained from fetuses of different gestational ages (8–14 weeks and 36–40 weeks), (2) in testicular bioptic specimens obtained from 2 prepubertal boys who died from road accidents, and (3) in testicular bioptic specimens obtained from adult males undergoing testicular biopsy for suspected abstructive azoospermia in whom a normal testicular morphology was found

RESULTS

Light microscopy

In the 8- to 14-week-old fetal testis a positive immunostaining was found mainly in the interstitial cells whereas nearly no staining could be observed in the germ cords In preterm fetuses (38 weeks of gestation) the immunostaining was present mainly in Sertoli cells and only in a few interstitial cells [11]

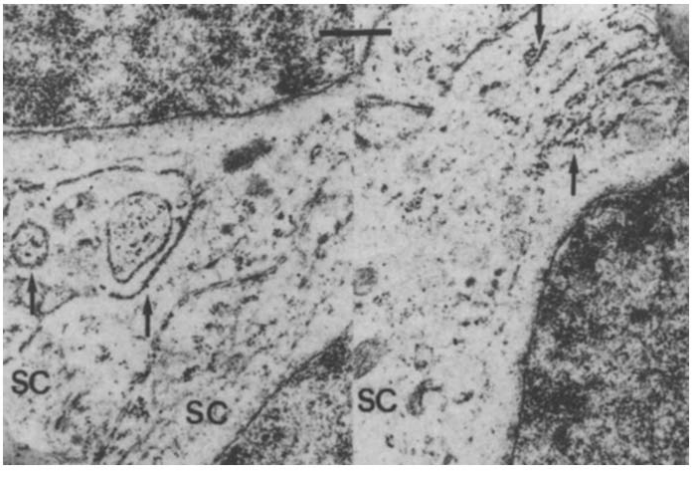

Fig 2 Immunogold localization of α inhibin in the rough endoplasmic reticulum of 3 Sertoli cells (SC) Bar = 1 3 μm

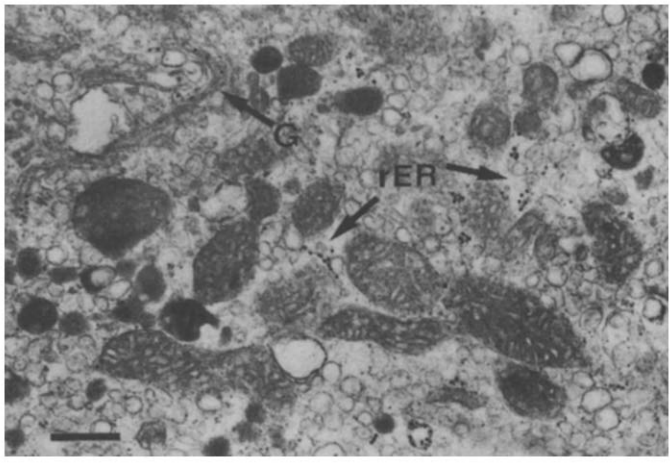

Fig 3 Immunogold labeling of α inhibin in the rough endoplasmic reticulum (rER) and in the Golgi apparatus (G) of a Leydig cell Bar = 0 75 μm Taken from Ref [11] with permission

In the prepubertal testis the immunostaining was present in the Sertoli cells but could not be found in the interstitial cells [11]

In the adult human testis the immunostaining was present not only in Sertoli cells, but also in spermatocytes (Fig 1) and in some Leydig cells

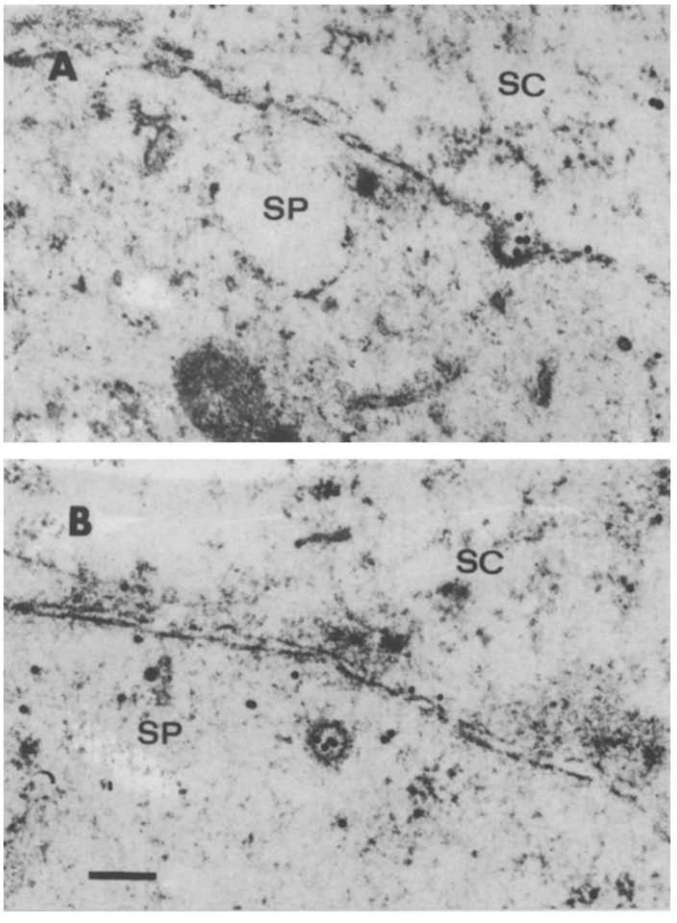

Fig 4 (A) Immunogold labeling of α inhibin in a coated pit of a spermatocyte (SP) (B) Immunogold labeling of α inhibin in a coated vesicle of a spermatocyte (SP) SC = Sertoli cell, SP = spermatocyte Bar = 0 18 μm

Electron microscopy

In the adult human testis gold labeling was found mainly in the cytoplasm of the Sertoli cells both in the rough endoplasmic reticulum (Fig 2) and in the Golgi cisternae A similar pattern of gold labeling was also found in some Leydig cells (Fig 3). In some spermatocytes gold labeling was found in coated pits and vesicles (Fig 4)

DISCUSSION

The presence of a positive α inhibin immunostaining in the interstitial fetal cells of 8- to 14-week-old fetuses suggests the possibility that, in this period of fetal life, the interstitial cells not only secrete testosterone, but also produce and/or store α inhibin The reduced immunoreactivity found in interstitial cells in preterm fetuses and the lack of immunoreactivity found in prepubertal interstitial cells shows that this phenomenon is coincident with the periods of reduced functional steroidogenetic activity of these cells

The immunohistochemical results we obtained in the human adult testis suggest that both Sertoli and Leydig cells can produce α inhibin molecules and are in agreement with the results of a similar histoimmunochemical study published recently [12] The positive immunostaining for α inhibin we found in some spermatocytes had not been previously reported, but was confirmed by the electron microscopy evidence of receptor-mediated internalization of α inhibin in these cells This finding suggests that inhibin can act on germ cells by a paracrine mechanism as can other Sertoli cell proteins [4]

However the concept that germ cells are the preferential target of Sertoli cell proteins has probably to be reconsidered for instance in the case of TF, a protein which we suggested was transferred from the Sertoli to the germ cells by a receptor-mediated endocytosis mechanism [4], we recently observed, using an *in situ* hybridization technique with a cDNA probe for human TF mRNA, that the anatomical site of TF expression in the adult human testis is not only the cytoplasm of Sertoli cells, as recently reported in the rat [13], but also the cytoplasm of developing germ cells

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REFERENCES

1 Griswold M D Protein secretions of Sertoli cells *Int Rev Cytol* **110** (1988) 133–156
2 Vannelli G B, Orlando C, Barni T, Natali A, Serio M and Balboni G Immunostaining of transferrin and transferrin receptor in human seminiferous tubules *Fert Steril* **45** (1986) 536–541
3 Vannelli G B, Barni T, Orlando C, Natali A, Serio M and Balboni G Insulin-like growth factor-I (IGF-I) and IGF-I receptor in human testis an immunohistochemical study *Fert Steril* **49** (1988) 666–669
4 Forti G, Barni T, Vannelli G B, Balboni G C, Orlando C and Serio M Sertoli cell proteins in the human seminiferous tubule *J Steroid Biochem* **32** (1989) 133–144
5 Gonzales G F, Risbridger G P and de Kretser D M *In vitro* synthesis and release of inhibin in response to FSH stimulation by isolated segments of seminiferous tubules from normal adult male rats *Molec Cell Endocr* **59** (1988) 179–185
6 Meunier H, Rivier C, Evans R M and Vale W Gonadal and extragonadal expression of inhibin α, βA and βB subunits in various tissues predicts diverse functions *Proc Natn Acad Sci U S A* **85** (1988) 247–251
7 Toebosch A M W, Robertson D M, Trapman J, Klaassen P, de Paus R A, de Jong F H and Grootegoed J A Effects of FSH and IGF-I on immature Sertoli cells inhibin α and β subunit mRNA levels and inhibin secretion *Molec Cell Endocr* **55** (1988) 101–105
8 Risbridger G P, Clements J, Robertson D M, Drummond A E, Muir J, Burger H G and de Kretser D M Immuno and bioactive inhibin and inhibin α subunit expression in rat Leydig cell cultures *Molec Cell Endocr* **66** (1989) 119–122
9 McLachlan R I, Matsumoto A M, Burger H G, de Kretser D M and Bremner W J The relative role of follicle stimulating hormone and luteinizing hormone in the control of inhibin secretion *J Clin Invest* **82** (1988) 880–884
10 Drummond A E, Risbridger G P and de Kretser D M The involvement of Leydig cells in the regulation of inhibin secretion by the testis *Endocrinology* **125** (1989) 510–515
11 Vannelli G B, Balboni G C, Barni T, Orlando C, Serio M and Forti G Immunohistochemical localization of inhibin α subunit in the fetal, prepubertal and adult human testis In *Molecular and Cellular Biology of Reproduction* (Edited by G Spera, L Gnessi, A Fabbrini and C W Bardin) Raven Press, New York (1992) In press
12 Bergh A and Cajander S Immunohistochemical localization of inhibin-α in the testes of normal men and in men with testicular disorders *Int J Androl* **13** (1990) 463–469
13 Morales C, Hugly S and Griswold M D Stage dependent levels of specific mRNA transcripts in Sertoli cells *Biol Reprod* **36** (1987) 1035–1046