Expression and localization of inhibin α , inhibin/activin β A and β B and the activin type II and inhibin β -glycan receptors in the developing human testis

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Inhibins and activins have roles in the regulation of cell proliferation and differentiation in a variety of tissues. This study investigated the distribution of the three inhibin/activin subunits (α , β A and β B) and their receptors in the human testis between week 13 and week 19 of gestation using RT-PCR and immunohistochemistry. mRNA for all three subunits and for the activin type II receptors ActRIIA and ActRIIB was detected at all stages of gestation examined. Sertoli cells showed intense immunostaining for the α subunit and some staining for the β B subunit, whereas only the β B subunit was detected in gonocytes. No β A subunit staining was detected within the tubules. All three subunits were localized to interstitial Leydig cells. Cells of the rete testis and the epididymal epithelium also showed immunostaining for β B; however, staining for the other

subunits was weak or absent. Peritubular cells showed intense immunostaining for the β -glycan inhibin receptor, which was also localized to interstitial cells, but was not detected within the tubular compartment, rete testis or epididymal epithelium. ActRIIA was detected in gonocytes and in interstitial cells; ActRIIB was distributed widely. These data indicate that fetal Leydig and Sertoli cells have the potential to produce both activins and inhibins, whereas gonocytes may produce only activin B. The distribution of activin and inhibin receptors implies that the intratubular compartment and developing duct system are sites of action of activin B but not inhibin at this stage of development, whereas both activins and inhibins may be involved in the development and function of the peritubular and interstitial cells.

Introduction

In humans, differentiation of the testis is evident from week 8 of gestation, when the testis comprises cords containing Sertoli cells surrounded by an interstitium that includes Leydig cells. Primordial germ cells, originating from the extraembryonic mesoderm of the yolk sac, increase in number by mitosis during migration and become associated with the cells within the gonadal ridges at about week 6 (Byskov and Høyer, 1996). During fetal life, male germ cells continue to proliferate up to approximately week 22 of gestation (Hilscher, 1991). Interstitial Leydig cells also undergo differentiation and proliferation during this period (Voutilainen, 1992; Murray *et al.*, 2000). This period of development is believed to be central to the establishment of adult testicular function and is a time of potential vulnerability to external influences (Sharpe, 2001). A number of paracrine factors involved in the control of proliferation and differentiation of the various types of cell have been identified, including members of the transforming growth factor β (TGF- β) family, which regulate gonocyte survival in the developing testis (reviewed by Josso and di Clemente, 1999; Olaso and Habert, 2000).

Activins and inhibins are members of the TGF- β family, initially characterized by their ability to regulate FSH secretion from the pituitary. The component α and β subunits can be combined into biologically active $\alpha\beta$ inhibin or $\beta\beta$ activin dimers, with generally opposing functional effects, and are produced in both the gonad and the pituitary (Burger and Igarashi, 1988; Lin *et al.*, 1989; Chen, 1993; Mather *et al.*, 1997). In men, the most clearly established physiological role is that of testis-derived inhibin B in the feedback regulation of FSH secretion (Illingworth *et al.*, 1996; Hayes *et al.*, 1998) but it is likely that both activin and inhibin have local effects within the testis (Mather *et al.*, 1997; Ethier and Findlay, 2001). Investigation of the expression of inhibin/activin subunits in the developing human testis has demonstrated that the α subunit is expressed in both the Leydig cells and in the Sertoli cells of the tubular compartment (Rabinovici *et al.*, 1991; Eramaa *et al.*, 1992; Majdic *et al.*, 1997; Roberts, 1997), similar to expression in other species (Majdic *et al.*, 1997; Jarred *et al.*, 1999), but there are conflicting data regarding the presence and localization of expression of the β subunits. *In situ* hybridization demonstrated expression of the β A subunit in the interstitium and the β B subunit in the tubules (Roberts, 1997). In contrast, both β A and β B subunits were localized to the interstitium but were not detected in the tubules in a study involving

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immunohistochemistry (Rabinovici *et al.*, 1991), whereas in another study the β B subunit was detected in Sertoli and Leydig cells, but β A was not detected (Majdic *et al.*, 1997). There also appear to be species differences in the localization of the subunits: in the ovine fetal testis, Sertoli cells were demonstrated to contain β A and β B subunits by immunohistochemistry and *in situ* hybridization (Jarred *et al.*, 1999), whereas in the rat fetal testis immunostaining for the β B subunit was intense but no immunostaining for β A was detected (Majdic *et al.*, 1997). There are limited data on the potential functional effects of activin and inhibin during testicular development, although activin has been shown to inhibit cell proliferation in the rat fetal testis (Kaipia *et al.*, 1994) and to regulate gonocyte and Sertoli cell proliferation in the rat postnatal testis (Boitani *et al.*, 1995; Meehan *et al.*, 2000).

Activin signalling is mediated by binding to the type II receptor serine kinases ActRIIA or ActRIIB, which recruit and phosphorylate the type I/ALK4 receptor with subsequent modulation of gene expression via Smad protein activation (Massagué, 1998). The mechanism of inhibin signalling has remained less certain, although recently two inhibin receptors, β -glycan (Lewis *et al.*, 2000) and p120 (Chong *et al.*, 2000), have been characterized, which may either block activin signalling or mediate specific signalling pathways (Matzuk, 2000; Roberston *et al.*, 2000). β -Glycan was immunolocalized to Leydig cells in the rat testis (Lewis *et al.*, 2000) and p120 to both Leydig and Sertoli cells. Radiolabelled inhibin also binds to Leydig cells (Krummen *et al.*, 1994), whereas activin receptors are expressed by Sertoli cells, spermatocytes and round spermatids (de Winter *et al.*, 1992; Kaipia *et al.*, 1992; Krummen *et al.*, 1994). Activin receptors are also expressed in the seminiferous tubules of the rat fetal testis (Roberts and Barth, 1994). Therefore, there appear to be cell-specific patterns of distribution of activin and inhibin receptors within the testis, but the distribution of these receptors in the developing human testis is unknown.

The aim of the present study was to explore the expression and distribution of inhibin/activin subunits in the human fetal testis during the period of proliferation of both somatic and germ cells after testicular cord formation. The localization of activin receptors ActRIIA and ActRIIB and of the inhibin receptor β -glycan was also investigated to identify potential sites of action of these regulatory factors during this period of development.

Materials and Methods

Tissues

Human fetal testes were obtained after medical termination of pregnancy. Women gave written consent according to national guidelines (Polkinghorne, 1989) and the study was approved by the Lothian Paediatrics/Reproductive Medicine Research Ethics Sub-Committee. Termination of pregnancy was induced by treatment with mifepristone (200 mg orally) followed 48 h later by prostaglandin E1

analogue (Gemepro; Beacon Pharmaceuticals, Tunbridge Wells) 1 mg per 3 h per vaginam. None of the terminations was for reasons of fetal abnormality, and all fetuses appeared morphologically normal. Gestational age was determined by ultrasound examination before termination and confirmed by subsequent direct measurement of foot length. Ten specimens were used for this study.

Testes were dissected free and either fixed for immunohistochemical analysis or snap frozen and stored at -70°C . Fixation was carried out in Bouin's fixative for 5 h, followed by transfer to 70% ethanol before processing into paraffin wax using standard methods.

Isolation of RNA and synthesis and amplification of cDNA

Total RNA was isolated from fetal testis using the RNeasy mini kit (Qiagen, Crawley) and treatment with DNAase (Gibco, Paisley). Reverse transcription using a first strand cDNA synthesis kit (Roche Diagnostics, Lewes) was followed by PCR using 1 μl cDNA samples and *Taq* DNA polymerase (AGS Gold; Hybaid, Ashford) as described by Robinson *et al.* (2001). Specific primers for each subunit and receptor are presented (Table 1). For each PCR, two control tubes were run in parallel, one in which water replaced the RNA and a second in which reverse transcriptase (RT -ve) was omitted, to ensure that there was no genomic DNA contamination. All pairs of primers were designed to span an intron to ensure genomic DNA was not amplified.

Immunohistochemistry

Sections (5 μm) were mounted on slides coated with 3-aminopropyl triethoxy-silane (TESPA; Sigma, Poole) and dried overnight at 50°C before processing for immunohistochemistry as described by Robinson *et al.* (2001). In brief, after inhibition of endogenous peroxidase activity by incubation in 3% (v/v) H_2O_2 in methanol, slides were blocked for 30 min in the appropriate normal serum (Diagnostics Scotland, Carlisle). Sections were then blocked with avidin and biotin (Vector, Peterborough). The following mouse monoclonal primary antibodies to the inhibin/activin subunits were used: α subunit (173.9k), β A subunit (E4), β B subunit (C5 and 12/13) (all were gifts from N. P. Groome, Oxford Brookes University). The primary antibodies were applied at concentrations of 1 $\mu\text{g ml}^{-1}$ (173.9k and E4), 5 $\mu\text{g ml}^{-1}$ (C5) and 4 $\mu\text{g ml}^{-1}$ (12/13) in the appropriate serum at 4°C overnight. Goat antibody to β -glycan (Santa Cruz Biotechnology, Santa Cruz, CA) was used at a dilution of 1:25. Rabbit antisera to ActRIIA and ActRIIB were kindly donated by R. Heldin (Ludwig Institute for Cancer Research, Uppsala, Sweden) and used at a dilution of 1:600. Rabbit antiserum to 3β -hydroxysteroid dehydrogenase (3β -HSD) (a gift from I. J. Mason, University of Edinburgh) was used at a dilution of 1:1000.

Sections were subsequently incubated with rabbit anti-mouse (Diagnostics Scotland), rabbit anti-goat (Dako,

Table 1. Sequences of PCR primers used for detection of inhibin/activin subunits and the activin receptors ActRIIA and ActRIIB in human fetal testis

Primers	5'	3'
Inhibin α	TGAGGGCCCTGTTCTTGATG	CTGGCGGCTGCGTGTATGCTG
Inhibin/activin β A	GAAGTTATGGAGCAGACCTCGG	TTGCCATCACACTCCAAGCC
Inhibin/activin β B	GCCAGGAGCGCGTTCCGAAATC	CCGCTCGCCCCGCTCAAACAAG
ActRIIA	GCAAAATGAATACGAAGTCTA	GCACCTCTAATACCTCTGGA
ActRIIB	CAACTTCTGCAACGAGCGCTT	GCGCCCCGAGCCTTGATCTC

Cambridge) or goat anti-rabbit (Santa Cruz Biotechnology) biotinylated secondary antibody, as appropriate, at a dilution of 1:500, followed by avidin–biotin–horseradish peroxidase-linked complex (Dako), according to the manufacturer's instructions. Bound antibody was visualized using 3,3'-diaminobenzidine tetra-hydrochloride (Dako). For negative controls, IgG or non-immune serum, as appropriate, was used in place of the primary antibody. Positive controls (human placenta and fetal membranes) were also run in parallel.

All sections were counterstained with haematoxylin, dehydrated, mounted and visualized by light microscopy. Images were captured using an Olympus Provis microscope (Olympus Optical Co., London) equipped with a Kodak DCS330 camera (Eastman Kodak).

Results

RT-PCR

Amplicons of expected sizes corresponding to the inhibin/activin subunits and the activin ActRIIA and ActRIIB receptors were amplified from cDNA derived from testes at all stages of gestation in the range week 13 to week 19 (Fig. 1).

Immunohistochemistry

Inhibin/activin subunits. Immunostaining for the α subunit was detected in both the interstitium and seminiferous tubules in all specimens examined (Fig. 2a–d). Most Leydig cells within the interstitium were immunostained, but staining was more intense in the Sertoli cells (Fig. 2c). No immunostaining of gonocytes or peritubular cells was detected at any stage of gestation investigated. The epithelial cells of the rete testis (Fig. 2a,d) and both the epithelium and stromal tissue of the adjacent epididymis (Fig. 3a,b) were also immunonegative.

The β A inhibin/activin subunit was localized to the cytoplasm of interstitial Leydig cells (Fig. 2e–h). No intratubular immunostaining of either the Sertoli cells or gonocytes was seen, and peritubular cells too were immunonegative. The rete testis (Fig. 2h) and epithelial cells of the epididymis (Fig. 3c,d) showed very weak immunostaining.

The β B subunit was also localized to the cytoplasm of interstitial Leydig cells, similar to the α and β A subunits (Fig. 2i–l); however, strong nuclear staining of interstitial cells

Fig. 1. Examples of RT-PCR analysis of samples extracted from human fetal testis at weeks 13, 16 and 19 of gestation, amplified using primers specific for ActRIIA, ActRIIB, inhibin α , inhibin/activin β A subunit and inhibin/activin β B subunit. RT-: samples in which reverse transcriptase was omitted. The size of each amplicon is indicated on the right.

was also seen (Fig. 2j,k). Nuclear staining was detected using two monoclonal antibodies (C5 and 12/13) and very similar results were obtained. Although not all interstitial cells showed either cytoplasmic or nuclear staining, most cells showed staining in both areas. This was particularly evident in interstitial cells with large round nuclei, whereas other interstitial cells with oval or irregular nuclei generally did not show cytoplasmic or nuclear staining; the cells with large round nuclei were also immunopositive for β HSD (data not shown), indicating that they were fetal Leydig cells. Nuclear staining was not seen in other types of cell. Immunostaining was also detected in the tubular compartment, particularly in Sertoli cells, and gonocytes were also immunopositive (Fig. 2k). In contrast to the pattern of immunostaining for the α and β A subunits, the cells of the rete testis showed very strong staining for the β B subunit (Fig. 2i,l), as did the epithelial cells of the epididymis (Fig. 3e,f).

Fig. 2. Immunohistochemical localization of (a–d) inhibin α subunit, (e–h) inhibin/activin β A subunit and (i–l) inhibin/activin β B subunit to fixed tissue sections from human fetal testis. The top row (a,e,i) illustrates the distribution of the subunits at low magnification. The second (b,f,j) and third (c,g,k) rows illustrate the distribution of the subunits in the gonocytes, Sertoli cells and peritubular cells of the testicular tubules, and in the interstitium at higher magnification. The bottom row (d,h,l) illustrates the distribution of the subunits in the rete testis. Antibody C5 was used for detection of β B subunit, with the exception of the section shown in (k) in which 12/13 was used. gc: gonocytes; in: interstitium; p: peritubular cells; r: rete testis; s: Sertoli cells; t: tubule. Scale bars represent (a,e,i) 500 μ m, (b,d,f,h,j,l) 50 μ m and (c,g,k) 20 μ m.

Fig. 3. Immunohistochemical localization of inhibin/activin subunits and receptors in human fetal epididymis: (a,b) inhibin α subunit, (c,d) inhibin/activin βA subunit, (e,f) inhibin/activin βB subunit, (g,h) activin receptor ActRIIA, (i,j) activin receptor ActRIIB and (k,l) β -glycan. Scale bars represent (a,c,e,g,i,k) 50 μ m and (b,d,f,h,j,l) 20 μ m.

Fig. 4. Immunohistochemical localization of activin receptors (a–d) ActRIIA and (e–h) ActRIIB and of (i–l) β -glycan to fixed tissue sections of human fetal testis. The top row (a,e,i) illustrates the distribution of the subunits at low magnification. The second (b,f,j) and third (c,g,k) rows illustrate the distribution in the gonocytes and peritubular cells of the testicular tubules and in the interstitium at higher magnification. The bottom row (d,h,l) illustrates the distribution of the subunits in the rete testis. epi: epididymis; gc: gonocyte; in: interstitium; p: peritubular cells; r: rete testis; t: tubule. Scale bars represent (a,e,i) 500 μ m, (b,d,f,h,j,l) 50 μ m and (c,g,k) 20 μ m.

No marked change in immunostaining for the three inhibin/activin subunits was seen throughout the period of gestation examined, from week 13 to week 19. In all cases negative controls showed no immunostaining (data not shown).

Activin and inhibin receptors. The distribution of the ActRIIA receptor showed a gradient within the testis in addition to a specific cellular localization (Fig. 4a–d). In the interstitium, many cells showed intense immunostaining (Fig. 4b). The intense staining was particularly evident towards the periphery of the testis, with positive but reduced immunostaining centrally and very weak staining in the rete testis (Fig. 4d). Within the tubular compartment, ActRIIA was localized predominantly to the gonocytes, although some staining of Sertoli cells was observed (Fig. 4c). Peritubular cells were also weakly immunopositive. The epithelial cells of the epididymis showed strong immunostaining for ActRIIA (Fig. 3g,h), although this varied among cells within a duct cross-section: cells showing intense staining were observed adjacent to cells showing more moderate staining.

ActRIIB immunostaining was also distributed widely within the fetal testis (Fig. 4e–h). Positive immunostaining was seen in both the interstitial cells, and in both gonocytes and Sertoli cells within the tubules. The peritubular cells were also a site of ActRIIB localization. In contrast to the ActRIIA receptor, ActRIIB was localized to the cells of the rete testis (Fig. 4h) and was also observed in the epithelial cells of the epididymis (Fig. 3i,j).

β -Glycan immunostaining was intense in the peritubular cells at all stages of gestation examined (Fig. 4i–k). Immunostaining was also present in the interstitium (Fig. 4j,k). No immunostaining was present in any cells in the intratubular compartment or in the rete testis (Fig. 4j–l). However, immunopositive cells were observed in the epididymis (Fig. 3k,l), but in contrast to the distribution of the activin receptor monomers, β -glycan immunoreactivity was localized to the stromal cells surrounding the epididymal ducts but was not observed in the epithelial cells.

Discussion

The data from the present study extend our understanding of the role of the activin/inhibin system during human testicular development, demonstrating differential patterns of distribution of the three activin/inhibin subunits and their receptors, indicating the ability of specific types of cell to synthesize, and be regulated by, the various activins and inhibins. The period of development investigated in the present study is characterized by high steroidogenic activity and proliferation and development of Sertoli cells and gonocytes (Hilscher, 1991; Voutilainen, 1992); thus, its regulation is central to subsequent reproductive function.

Within the developing testicular tubules, gonocytes expressed the β B subunit, but not other subunits, and both the ActRIIA and ActRIIB receptors, whereas Sertoli cells

expressed both α and β B subunits. Sertoli cells also expressed both activin type II receptors, although expression of ActRIIA was weak. Neither type of cell expressed the β -glycan inhibin receptor. These findings imply that gonocytes can synthesize only activin B, and may be responsive both to autocrine production of activin B and to paracrine secretion of activins produced by Sertoli cells. Activin regulates gonocyte and Sertoli cell proliferation in the rat neonatal testis (Boitani *et al.*, 1995; Meehan *et al.*, 2000) and increases spermatogonial proliferation at later stages of development (Mather *et al.*, 1990). Disruption of the β A subunit gene resulted in neonatal lethality due to suckling defects (Matzuk *et al.*, 1995a), whereas deletion of the β B subunit had no apparent effect on spermatogenesis (Vassalli *et al.*, 1994). Substitution of the β B subunit for the β A subunit (β B knock-in) rescued the β A lethality, but the subunit acted as a hypomorphic allele with dosage effects on gonadal development, including delayed onset of spermatogenesis similar to that seen with ActRIIA deficiency (Matzuk *et al.*, 1995b; Brown *et al.*, 2000), revealing a role for activin A during testicular development. Although effects on FSH secretion complicate interpretation of some of these phenotypes, double knockouts to both FSH β and ActRIIA confirm a role for this receptor pathway in the regulation of the number of germ cells within the testis (Kumar *et al.*, 2001). Conversely, overexpression of the β A subunit led to disrupted spermatogenesis (Tanimoto *et al.*, 1999), although this was a postnatal rather than developmental abnormality, and potential effects at earlier developmental stages were not described. Therefore, activin may be of importance in the development of testicular tubules in men. However, species differences in β subunit expression are apparent. The rat gonocyte expresses the β A subunit (Mather *et al.*, 1997; Meehan *et al.*, 2000), which was not detected in the intratubular compartment in the present study. Similarly, β A expression has been demonstrated in the ovine gonocyte (Jarred *et al.*, 1999). Previous studies on human fetal testis have also indicated the absence of β A mRNA and protein in the tubular compartment, as revealed by *in situ* hybridization (Roberts, 1997) and by immunohistochemistry (Majdic *et al.*, 1997). The presence of β B mRNA and protein within the tubules has been demonstrated (Majdic *et al.*, 1997; Roberts, 1997), although no specific comment was made regarding expression by gonocytes.

A similar species difference is apparent in the expression of inhibin/activin subunits by the rete testis. Limited data are available, but in sheep fetuses the rete testis expresses only the β A subunit (Jarred *et al.*, 1999), whereas in the present study expression of the β B subunit, but not the α or β A subunits, was demonstrated. In these species, the situation is similar in the adult: in rams, the circulating form of inhibin is inhibin A (McNeilly *et al.*, 2000), whereas in humans inhibin B, but not inhibin A, is present (Illingworth *et al.*, 1996; Anderson *et al.*, 1997). The expression of the β B subunit, but not the α subunit, and of the ActRIIB

receptor by the rete testis indicates that activin B, but not activin A or inhibin, is involved in the development of this structure in humans. The cells of the rete testis are believed to be of mesonephric origin and may contribute to both Leydig and Sertoli cell precursors (Wartenberg, 1989).

In addition to the production of activins, the presence of the α subunit indicates that fetal Sertoli cells may also produce inhibin B, consistent with the presence of inhibin B in the fetal circulation (Wallace *et al.*, 1997). The Sertoli cell is believed to be the source of circulating inhibin B in adult men (Anawalt *et al.*, 1996; Illingworth *et al.*, 1996), and secretion of inhibin B is thought to be regulated by both FSH and the germ cell complement (Anderson *et al.*, 1997; Andersson *et al.*, 1998). It has been suggested that expression of the β B subunit is lost during Sertoli cell maturation during puberty, and that in the adult it is expressed by primary spermatocytes and spermatids (Andersson *et al.*, 1998). Therefore, testicular production of inhibin may contribute to the regulation of FSH secretion at this stage in development, which in turn may contribute to Sertoli cell and gonocyte proliferation (Orth, 1984; Meehan *et al.*, 2000). Inhibin inhibits spermatogonial proliferation in adult rats (van Dissel-Emiliani *et al.*, 1989), but effects on fetal tissue have not been reported. A major role of inhibin in the regulation of Sertoli cell proliferation is also indicated by the results of targeted disruption of the α subunit gene, which resulted in the development of testicular tumours, probably arising from Sertoli cells (Matzuk *et al.*, 1992).

The marked localization of the β -glycan receptor to the peritubular cells may indicate that these cells are a target for Sertoli cell-derived inhibin, but it is likely that there are other forms of inhibin receptor (Chong *et al.*, 2000; Roberston *et al.*, 2000) not investigated in the present study that may be present within the tubular compartment. Both β -glycan and inhibin binding sites were localized to the Leydig cells rather than to the tubular compartment in rats (Krummen *et al.*, 1994; Lewis *et al.*, 2000). Peritubular cells were also shown to express the β B subunit in the present study. Expression of both ActRIIA and ActRIIB was also apparent, although at a low level. These findings are consistent with the demonstration of activin secretion and activin receptor mRNA in cultured rat peritubular cells (de Winter *et al.*, 1994). Peritubular cells are also the site of expression of androgen receptors and neurotrophin in the human fetal testis (Russo *et al.*, 1999; Murray *et al.*, 2000); thus, these cells may have a significant role in testicular development, potentially contributing to both tubular and interstitial function.

Leydig cells expressed all three inhibin/activin subunits, in broad agreement with previous studies (Rabinovici *et al.*, 1991; Eramaa *et al.*, 1992; Majdic *et al.*, 1997; Roberts, 1997). Interstitial cells were also shown to express the β -glycan inhibin receptor and both forms of activin receptor; thus, inhibin and activins may have roles in the autocrine or paracrine regulation of fetal Leydig cell steroidogenesis and proliferation. Inhibin is produced by Leydig cells in adult rats (Risbridger *et al.*, 1989) and both inhibin and activin

may regulate steroidogenesis (Hsueh *et al.*, 1987). Gonadotrophin regulation is indicated by stimulation of secretion of the α subunit from human fetal testis by hCG (Rabinovici *et al.*, 1991), also seen in men (Anawalt *et al.*, 1996; Kinniburgh and Anderson, 2001). An unexpected finding was the localization of the β B subunit to the nuclei of some interstitial cells, using two separate monoclonal antibodies. Immunostaining with antiserum against 3 β -HSD labelled interstitial cells of similar morphology (data not shown), as described in both humans and other species (Jarred *et al.*, 1999; Murray *et al.*, 2000). The cells in which the nuclei stained for the β B inhibin/activin subunit therefore appear to be fetal Leydig cells. Nuclear staining was not seen in other types of cell or with other inhibin/activin subunits. Nuclear localization of the β B subunit has not been reported to date, although the β A subunit has been localized to nuclei of intermediate spermatogonia, spermatocytes and elongating spermatids at some stages of the spermatogenic cycle in rats (Bläuer *et al.*, 1999). In the study of Bläuer *et al.* (1999), a functional nuclear localization signal was identified within the inhibin β A subunit sequence by transient expression. Nuclear localization of other growth factors based on cleavage of specific motifs is well recognized; the nuclear and secreted forms have opposing effects of cell proliferation in some models (Kiefer and Dickson, 1995; Antoine *et al.*, 1997). The present finding requires confirmation, but may be a further mechanism of regulation of the action of inhibins and activins.

The pattern of expression of the activin/inhibin subunits and receptors was similar in the rete testis and the adjacent epididymis. Both tissues showed expression of the β B subunit (and weakly of the β A subunit), but not of the α subunit, and of ActRIIB, indicating a role for activin but not inhibin in the duct epithelium. However, stromal cells surrounding the epididymal ducts expressed β -glycan, although the potential source of inhibin in this site was unclear.

In conclusion, this study has demonstrated specific cellular localization of inhibin/activin α , β A and β B subunits, the β -glycan inhibin receptor and the activin type II receptors in the human fetal testis. These results are consistent with the involvement of activins in Sertoli cell and gonocyte proliferation and development in concert with FSH, whereas the major role of inhibins may be within the interstitium. The rete testis and peritubular cells may be additional sites of action of activin B and inhibin, respectively.

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