Immunocytochemical localization of hFSH as an index of Sertoli cell function in the human testis

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Abstract. The FSH receptor in the human testis has not been well characterized in vivo. Using an immunoperoxidase technique we have attempted the immunocytochemical localization of FSH in testicular tissue from patients with a variety of disorders including oligo- or azoospermia (N = 6), cryptorchidism (N = 3), and prostatic carcinoma (N = 3). Specific staining for hFSH was observed inside the seminiferous tubule, generally near the basal membrane in all except the cryptorchid patients. Specific staining was also localized in the luminal area of the seminiferous tubule. In most cases, FSH-positive cells were also found in the interstitium, with a minority of the cells being macrophages. The latter were more prevalent in the undescended testes and in orchiectomy specimens from patients with prostatic cancer. The pattern of FSH localization observed in this study probably represents receptor-bound hormone, and may reflect damage to the Sertoli cell and its tight junctions. Further study of the changes in receptor distribution as an indication of Sertoli cell malfunction, may be helpful in our understanding of human testicular disorders.

The Sertoli cell (SC) contains FSH receptors through which the physiological activities of the cell are regulated. It controls and maintains a tubular fluid environment optimal for spermatogenesis, and may also directly communicate with the neighbouring Leydig cell to modulate steroidogenesis locally (Sharpe 1984). The study of FSH receptors and of the factors modulating their availability may be important in our understanding of certain disorders. For example, cryptorchidism alters FSH receptors and results in an impaired production of androgen binding protein and inhibin in the rat (Jegou et al. 1983; Seethalakshmi & Steinberger 1983).

Immunocytochemical localization of FSH has been reported in rat and human pituitaries (Nakane 1970; Phifer et al. 1973) and in rat testis (Hon et al. 1983; Wahlstrom et al. 1983). However, there is only limited information on the status of FSH receptors in the human testis. Receptors for FSH have been detected in some infertile patients by means of in vitro binding studies (Namiki et al. 1984, 1986) that required relatively large amounts of tissue, not usually permissible in a biopsy. Therefore, the immunocytochemical technique provides a more practical approach in the study of human disorders. Utilizing this technique, Wahlstrom et al. (1983) reported positive LH staining in human Leydig cells, but weak FSH staining in some Sertoli cells'. Recently, the same group of investigators reported positive FSH staining in the SC of inguinal human testis (Hovatta et al. 1986). In the current study, we have attempted the immunocytochemical localization of FSH using the peroxidase-anti-peroxidase (PAP) technique in testicular tissue from patients with oligo- or azoospermia, cryptorchidism, and prostatic carcinoma.
Materials and Methods

Testicular tissue was obtained from 12 patients with a variety of disorders including oligo- or azoospermia (N = 6, age: 29–41 years), cryptorchidism (N = 3, age: 21–41 years), and prostatic carcinoma (N = 3, age: 59–79 years). None of the patients had received any hormonal treatment before testis biopsy or orchidectomy.

A small piece of testicular tissue was directly put into buffered formalin (Millonig's solution) in the operating room and transported to our laboratory where it was fixed overnight at 4°C and embedded in paraffin. Five micron sections were cut for light microscopy (haematoxylin and eosin) and immunocytochemical staining.

The method used for immunocytochemical staining for FSH using a specific rabbit antiserum against the β-subunit of human FSH (NIAMDD-anti-hFSH beta IC-1) was modified from the unlabelled peroxidase-antiperoxidase technique of Sternberger et al. (1970). To distinguish macrophages from other testicular cells, the adjacent section was stained for lysozyme (LZM) by the same immunoperoxidase method (Reitamo 1978) using rabbit anti-human LZM (Accurate Chemical & Scientific Corp, Westbury, NY). For sensitivity controls, various dilutions were employed (1:50 to 1:1000) to observe the intensity of staining and determine suitable dilutions to use. For specificity controls, the antiserum was replaced with normal rabbit serum (NRS) or incubated with purified hFSH (NIAMDD-hFSH-2, 300 nmol/l) or Pergonal (70 000 IU/l, Serono Laboratories, Randolph, MA). Cross-absorption of the primary antiserum with purified hLH (NIAMDD-I-1, 180 nmol/l) indicated no cross-reactivity. In order to see if any FSH molecules that are not bound or processed by their receptor are retained during the experimental procedures, epididymal tissue from a normal adult rat was processed similarly, and found to be completely devoid of specific staining.

After deparaffinization and rehydration of the sections, the sequence of incubations was as follows: 1) 1% hydrogen peroxide in cold methanol for 30 min to

Fig. 1.

Immunocytochemical staining of testicular FSH in a patient with oligospermia. Positive staining was noted in the area near the basal membrane of the seminiferous tubules (scale bar: 50 µm). Rabbit antiserum to the β subunit of hFSH was used at a dilution rate of 1:300. When primary antiserum was pre-absorbed with purified hFSH, all the staining disappeared.
destroy endogenous peroxidase, 2) 3% normal goat serum (NGS, Gibco Laboratories, Grand Island, NY) for 30 min to block nonspecific tissue binding sites, 3) 1:300 dilution of primary antisera overnight at 4°C, 4) 1:10 dilution of goat anti-rabbit immunoglobulin G (GAR, Cappel Labs, Malvern, PA) for 30 min at room temperature, 5) 1:25 dilution of horseradish PAP raised in rabbit (Accurate Chemical & Scientific Corp, Hicksville, NY) for 30 min at room temperature. After each incubation step, the sections were washed twice for 7 min with Tris-buffered (0.5 mol/l, pH 7.6) saline solution. Finally they were stained with a filtered solution of 3,3'-diaminobenzidine hydrochloride (0.83 mmol/l, Eastman Kodak Co, Rochester, NY) containing 0.03% of hydrogen peroxide for 15 min. In additional controls, GAR or PAP were replaced with NGS or NRS, respectively.

The distribution of the specific staining was classified into four different categories according to the stained area or cell type, viz seminiferous tubule-basal, seminiferous tubule-luminal, interstitial cells, and macrophages. Interstitial cells (ICs) were cells forming clusters in the interstitium between the tubules, and not distinguishable from the Leydig cells. Luminal staining in the tubule included staining within the cytoplasm of the SC, and staining of the luminal surface (presumably the apical portion of the SC membrane), cell debris in the lumen (presumably cytoplasmic extensions of the SC), and slough (partially degenerated germ cells at various stages of development).

Results

The histologic changes ranged from minimal to moderate degrees of hypospermatogenesis in patients with oligospermia to total absence of spermatogenesis in patients with cryptorchidism. One patient with azoospermia showed only SCs. Concomitantly, the oligospermic patients showed, at most, only mild thickening of the walls of the seminiferous tubules, whereas marked hyalinization of tubules was common in cryptorchidism. Intermediate changes in terms of decreases in spermatogenesis, and thickening of peritubular

Fig. 2.
Immunocytochemical staining of testicular FSH in an oligospermic patient with varicocele (scale bar: 50 µm). The majority of the specific staining in the basal area is in the spermatogonia (arrows).
tissue were seen in orchiectomy specimens for prostatic carcinoma. Some tubules from oligospermic patients with varicocele showed slough in the luminal area.

Among the 12 patients studied, specific immunocytochemical staining of FSH was found in the basal (8 patients) and luminal areas (12 patients) of the seminiferous tubules, as well as in interstitial areas (11 patients). Basal staining was observed in oligospermic patients (Fig. 1), but was absent in all 3 cases of cryptorchidism studied. On the other hand, in two of the oligospermic patients with varicocele, the majority of the basal staining was apparently localized in the spermatogonia (Fig. 2). Although specific staining was demonstrated in the luminal areas of the tubules in all cases, a precise cellular localization was not possible (Fig. 3). Specific positive staining was also found in the luminal slough in all three oligospermic testes with varicocele.

When primary antiserum for hFSHβ was pre-incubated with purified hFSH or Pergonal, all the staining for hFSH disappeared (figure not shown). When anti-LZM serum was used as primary antiserum, some of the hFSH positive cells in the interstitium were found to be macrophages. As shown in Fig. 3, macrophages could be differentiated from ICs, since macrophages stained more strongly, were larger, and were always seen as isolated cells, whereas ICs presented as aggregates.

In all three cases of cryptorchidism studied, we could not demonstrate FSH staining in the basal area of the seminiferous tubule. In contrast, staining in the luminal area seemed more abundant. Specific FSH staining in macrophages was detected in only two out of 6 cases of oligospermia or azoospermia, whereas 5 out of the 6 patients with prostatic carcinoma or cryptorchidism were positive. On the other hand, specific staining for other ICs was found in most of the patients studied regardless of the disorder.
Discussion

Specific FSH staining was demonstrated in all tissue specimens studied, and in 8 out of 12 patients, FSH was stained along the basal area of the seminiferous tubule (Fig. 1). Although direct evidence is lacking, this probably represents receptor-bound hormone in a normal distribution within the tubule, as free FSH is not likely to be retained following all the washes and incubations outlined above. Furthermore, epididymal tissue, which lacks FSH receptors, was processed in an identical manner and found to be completely devoid of specific staining. Specific staining localized exclusively in the basal portion of the SC membrane was reported in rats studied by autoradiography (Orth & Christensen 1977, 1978), and may be expected in view of the evidence for SC tight junctions (Setchell et al. 1976; Nagano & Suzuki 1976). In the human testis, Nagano & Suzuki (1976) showed that there were more than 40 parallel-oriented tight junctions located circumferentially around the surface of the SC near the base of the epithelium.

There are two possible explanations for the specific staining in the luminal area of the tubule: 1) The underlying pathological conditions may have caused a partial loss of the SC tight junction, thus allowing extratubular FSH to reach the receptor located in the apical portion of the SC membrane. 2) Luminal staining may represent stored or metabolized FSH that was internalized through specific receptors in the basal area of the SC membrane. Hon et al. (1983) reported specific immunocytochemical staining in the cytoplasmic vesicles of isolated SC from rat testis. In contrast, Hutson et al. (1977) reported specific staining only in acrosomes and intranuclear bodies of spermatids in normal adult rats.

Although the number of subjects included in our study is small, absence of basal staining in cryptorchidism suggests an impairment of SC function. Furthermore, microscopic examination of the cryptorchid tissue in our patients showed severe degenerative changes. This may explain the discrepancy between our finding and the recent report of Hovatta et al. (1986) of positive testicular FSH staining in 4 patients with unilateral cryptorchidism.

As noted above, a number of the cells observed in the interstitial area were anti-LZM positive, and could, therefore, be identified as macrophages. Wahlstrom et al. (1983) reported similar findings in the rat and human testis. Testicular macrophages possess high affinity FSH receptors and respond to FSH in vitro and in vivo (Yee & Hutson 1983, 1985a,b). However, it is yet to be determined whether FSH-regulated secretory products from testicular macrophages play any role in testicular function. The apparent increase in the number of macrophages found in cryptorchidism and in prostatic carcinoma as compared with the oligospermic group may be related to the degenerative changes found in cryptorchidism and prostatic carcinoma.

It was evident in our study that the majority of the positively stained cells in the interstitium did not represent macrophages. The fact that ICs stained positively with anti-hFSHβ serum which had been cross-absorbed with hLH is a puzzling finding. Wahlstrom et al. (1983) also reported specific FSH staining of ICs in the human and rat testis, and stated that these cells were indistinguishable from Leydig cells. Metaplasia of a SC to a Leydig cell has been postulated (Schulze 1984), and there are well recognized tumours (Sertoli-Leydig cell tumour in the ovary and gonadal stromal tumour in the testis) in which both cell types are found, suggesting a close relationship between the two cell types (Mostofi & Price 1973).

In conclusion, the current study demonstrates conclusively a positive immunocytochemical identification of FSH in human testis. The staining was localized not only near the basal membrane of the seminiferous tubules, but also in the luminal and interstitial areas. Testicular macrophages were stained specifically with anti-hFSH serum, and appeared to be more numerous in patients with cryptorchidism and prostatic carcinoma. Further studies are necessary to determine the significance of FSH staining in the luminal areas of seminiferous tubules, and to characterize the identity of FSH-binding ICs.

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