Immunohistochemical Localization of β-Endorphin in Hyperplastic Interstitial Tissue of the Human Testis

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The immunohistochemical localization of β-endorphin in the normal testis (two patients) and in the pathologic testis (two cases of Sertoli Cell Only Syndrome, two cases of Klinefelter Syndrome, two cases of post-orchitis tubular sclero-hialinosis) was investigated. No β-endorphin immunostaining was detected in the normal testis, while positive β-endorphin immunostaining has been observed in pathologic tissues. These results indicate that, as in animals, β-endorphin is present in human Leydig cells and may play a local role in regulating male reproductive function.

Key words: β-endorphin, Leydig cells, immunohistochemistry.

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The presence of β-endorphin and ACTH-like immunoreactivity has recently been reported in various animal and human tissues (Krieger et al, 1980). Peripheral physiologic functions of β-endorphin and other opioids have been postulated in the regulation of reproductive function (Tsong et al, 1982a). Immunohistochemical localization of β-endorphin and ACTH-like material has recently been reported in multiple sites of the male reproductive tract of the rat (Tsong et al, 1982a), as well as in the Leydig cells of the other animals (Tsong et al, 1982b). Persistence of such material, demonstrated by immunohistochemical and immunoassay techniques following hypophysectomy (Tsong et al, 1982a), presumably suggests a local production and paracrine function in the testis. Recently, opiate receptors have been described on rat Sertoli cells and inhibition of Sertoli cell activity by endogenous opioids has been reported (Fabbri et al, 1985). Although β-endorphin-like material has been detected in human semen (Fraioli et al, 1984), no data exist in the literature on the localization of opioid peptides in the human testis. We have investigated the immunohistochemical presence of β-endorphin in the normal interstitial tissue of the human testis and in those cases in which the number of Leydig cells is greatly increased relative to the tubular epithelium, such as in severe tubular atrophy, Klinefelter Syndrome and Sertoli Cell Only Syndrome.

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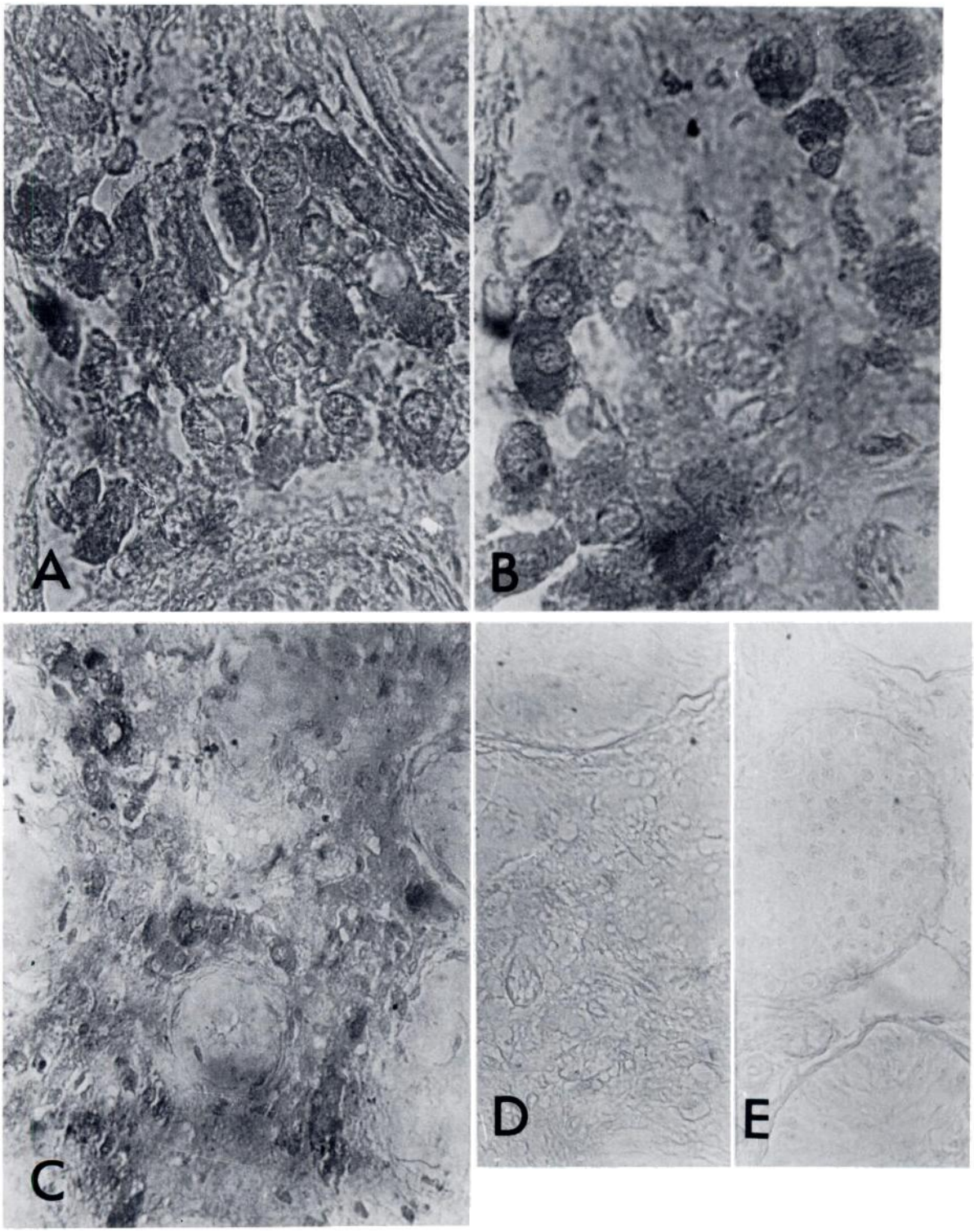

Fig. 1. Immunostaining with β-endorphin antibody of the Leydig cells in the Sertoli Cell Only Syndrome (A), in Klinefelter Syndrome (B), and in post-orchitis tubular sclero-hialinosis (C). (D) represents a control section of the case of Klinefelter Syndrome, while (E) is normal testicular tissue. Magnification: A: × 250; B: × 1000; C: × 250; D: × 500; E: × 250.

Materials and Methods

This study was performed on testicular biopsies obtained from patients with azoospermia due to a blockage of the deferent duct (normal testis: two patients) and with Leydig cell hyperplasia due to Sertoli Cell Only Syndrome (two cases), Klinefelter Syndrome (two cases), and post-orchitis tubular sclero-hialinosis (two patients). These patients had been carefully examined by means of clinical and endocrine evaluation before the testicular biopsy was performed.

LH, FSH and, testosterone (T) levels were assayed as previously described (Fraioli and Isidori, 1977).

The specimens were fixed in Bouin's fluid, embedded in paraffin, sectioned at 5 to 7 microns, and stained with the unlabeled antibody peroxidase-antiperoxidase technique (Sternberger et al, 1970). The antisera were used at a dilution of 1:300. Anti-human β-endorphin antiserum (provided by A.S.Liotta, Mount Sinai Hospital, New York, NY) raised against the C-terminal fragment of the molecule, cross-reacts with β-endorphin (1-31), Delta-endorphin (1-27), their acetylated forms, and β-lipotropin. Control slices were incubated with phosphate-buffered saline (PBS). Specificity controls of the immunoperoxidase reaction have been performed after preliminary treatment of the antibody with excess antigen (100 μg/ml diluted antiserum).

Results

In patients with Leydig cell hyperplasia, plasma T levels were low (< 350 ng/100 ml) while FSH and LH levels were higher than normal (> 20 I.U./ml). In patients with normal testis LH, FSH and T levels were in the normal range.

No β-endorphin immunostaining was detected in normal testicular tissue (Fig. 1E), while positive immunostaining was observed in hyperplastic interstitial tissue. β-endorphin immunoreactivity was strictly localized in the cytoplasm of many of the Leydig cells (Fig. 1 A-C). Negative immunoreactivity has been observed at the tubular wall. A poor, aspecific immunostaining has been reported in the cells of the seminiferous epithelium. No immunostaining was detected after incubation with the pretreated primary antiserum (Fig. 1D).

Discussion

Our results demonstrate that immunostainable β-endorphin-like material is present in the human testis and is localized in many, but not all, Leydig cells of hyperplastic testicular tissue. There are data in the literature (Margioris et al, 1983) regarding local synthesis of proopiomelanocortin-related peptides in the rat reproductive tract. Proopiomelanocortin-like gene expression has been described in rat Leydig cells and epididymis (Ching-Ling et al, 1984). Also, detailed analysis of the distribution of Leydig cell labeling has demonstrated that proopiomelanocortin positivity was more intense when the majority of the surrounding tubules were in stages IX to XII of the cycle of the seminiferous epithelium (Gizang-Ginsberg and Wolgemuth, 1985).

In our studies, β-endorphin immunoreactive Leydig cells were not observed in testicular specimens from the normal testis. These cells, however, were stained under conditions that enhance their number (Leydig cell hyperplasia). Since in the latter condition not all of the existing Leydig cells were immunoreactive, the question arises whether the lack of immunoreactive Leydig cells in the normal testis reflects a paucity of cells available for staining or reduced storage levels of β-endorphin. Interestingly, Negro-Vilar et al found that hCG is a potent stimulus to β-endorphin release from rat testicular tissue *in vitro* (Valenca and Negro Vilar, 1986). Also, Shaha et al (1984) observed that hCG treatment of immature hamsters and mice significantly increased to adult levels the number of Leydig cells that were immunopositive for β-endorphin. These results suggest that sustained LH levels could positively affect Leydig cell β-endorphin synthesis and release. This could also be the case in humans, since high plasma LH levels were observed in all patients with β-endorphin-immunopositive Leydig cells. To confirm this hypothesis, however, studies should be performed in which patients with normal Leydig cells receive one or several stimulatory doses of hCG and undergo testicular biopsy. The finding of β-endorphin in the human testis is novel and confirms observations reported in animal studies. Further qualitative and quantitative investigations are needed to clarify whether this peptide may have a role in regulating male reproductive function at the peripheral level in addition to the central effect (Grossman et al, 1981; Fraioli et al, 1982).

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