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Immunoexpression of Atrial Natriuretic Peptide in Patient with Seminoma

B. Nanova, A. Russinova***

**Dept. of Anatomy, Histology and Embryology, Medical University of Varna*
***Institute of Experimental Morphology and Anthropology, BAS, Sofia*

Atrial natriuretic peptide (ANP) is known to play an important role in mammalian spermatogenesis and steroidogenesis. However, the localization of ANP in human testis has been insufficiently examined. As well, it could be considered the significance of ANP in processes of degeneration and tumorogenesis. That's why in the current study we aimed to investigate immunohistochemically localization of ANP in patient with testicular tumor (seminoma) in terms of possible application of ANP as a marker for tumorogenesis. Material was received from testicular biopsy of a man with seminoma. Results showed positive immune reaction for ANP. Strong immunoexpression of ANP was observed in the cytoplasm of tumor cells. Therefore we could suggest a possible role for ANP in processes of germ cell degeneration and tumorogenesis.

Key words: immunohistochemistry, ANP, testis, seminoma, timorogenesis.

Introduction

Atrial natriuretic peptide (ANP) is known to play an important role in processes of spermatogenesis and steroidogenesis [1, 3 ,7]. Data about localization of ANP-receptor in testes of mice, rats and human were reported by several authors [2, 3, 4, 6]. In testis ANP is localized in the cytoplasm of germ, Sertoli and Leydig cells. According to B e x and C o r b i n [2] even low concentrations of ANP may lead to dose-dependent increase of testosterone secretion by Leydig cells. The presence of ANP in Leydig cells and it's functional receptors, suppose that ANP is an autocrine and/or paracrine regulator of these cells [5]. In the testis, atrial natriuretic peptide is observed in the wall of interstitial blood vessels, as well. The localisation and distribution of ANP in human testis is still not studied in detail, especially under pathological conditions. Natriuretic peptides and nitric oxide are involved in induction of apotosis of endothelial cells [8] and possible role of ANP in processes of degeneration and tumorogenesis can be suggested. In this respect we aimed to investigate morphological testicular changes in tandem with pattern of immunoexpression of ANP in a patient with seminoma in terms of possible application of ANP as a marker of tumorogenesis.

Material and Methods

Material for the current study was received from testicular biopsy No 147-R, from right testis of a man (32 years old) in Clinic of Urology in "St. Ana" hospital, Varna. Pathohistological diagnosis was "Seminoma testis dextra". Paraffin sections were prepared (7 μm) according to classical histological technique. Paraffin sections were processed also according to the classical immunohistochemical technoque using mouse monoclonal antibody 6C3 raised against human ANP that was generated and characterized in the Institute of Biology and Immunology of Reproduction – BAS [4]. Sections were deparaffinized and incubated with monoclonal anti-ANP antibody for 12 h. After washing with PBS sections were incubated with second biotinylated rabbit anti-mouse antibody (1:200). Avidin-biotin-peroxydase complex was applied and for visualization of immune reaction DAB was used.

Results

Our results from histological observation showed that at some places seminiferous tubules have saved structure. We also observed intact tubular wall at places with increasing amount of connective tissue (Fig.1).

Strong immune reaction for ANP in germ cells, and peritubular tissue was evident. Places affected by tumor process revealed increasing growth of tumor cells (Fig.2, 3). Tumor cells were forming nests and around them fine layers with lymphocytes were

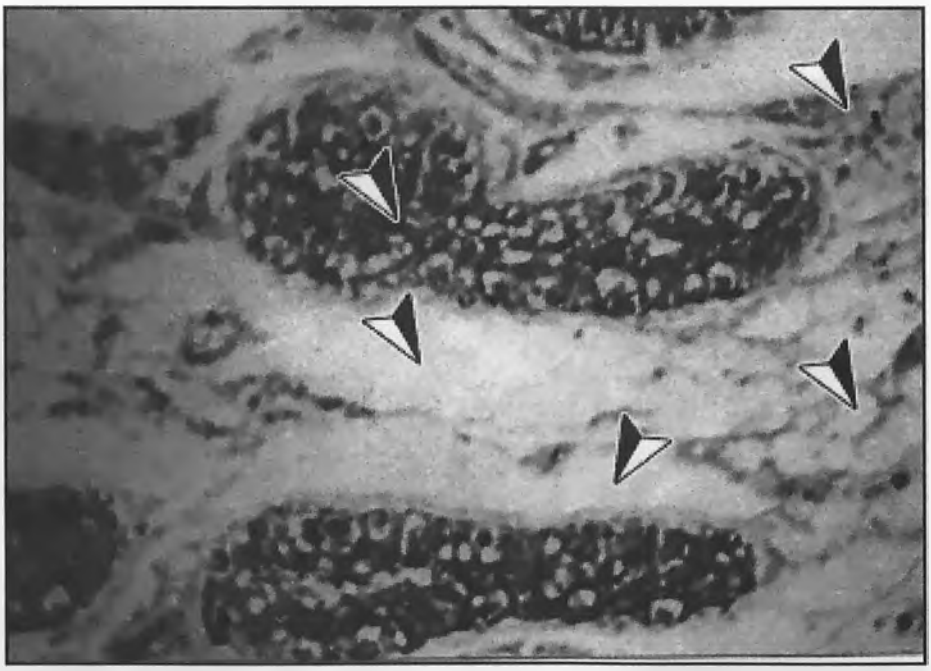

Fig. 1. Testicular biopsy. Seminoma. Seminiferous tubules with saved structure. Intensive collagenation. Strong immunoexpression for ANP in germinal epithelium and positive immune reaction in peritubular tissue. Monoclonal anti-ANP antibody (Microphoto × 200)

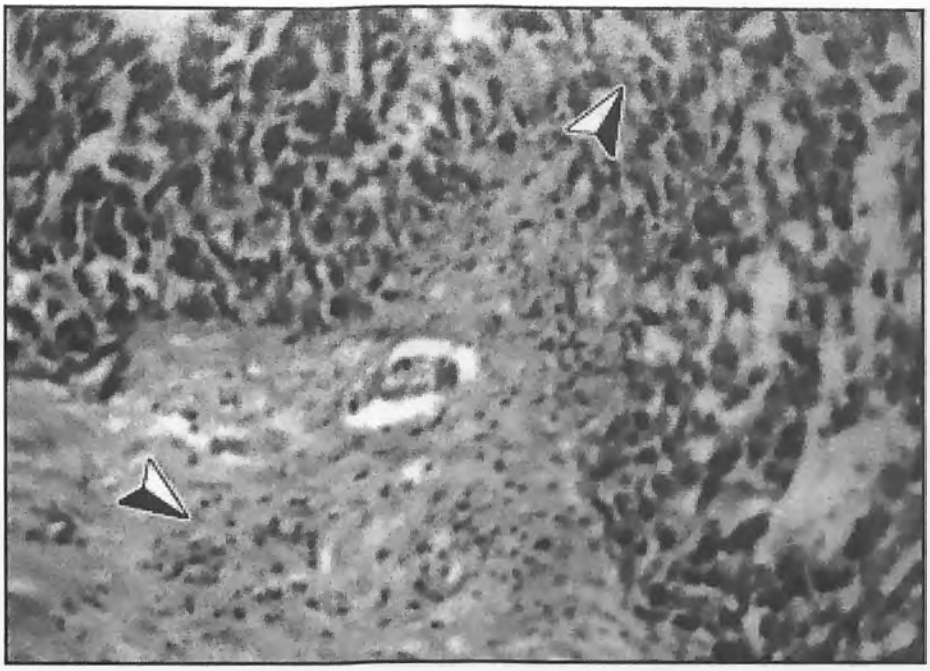

Fig. 2. Testicular biopsy. Seminoma. Places with increasing growth of tumor cells. Haematoxyline-eosine staining (Microphoto × 200)

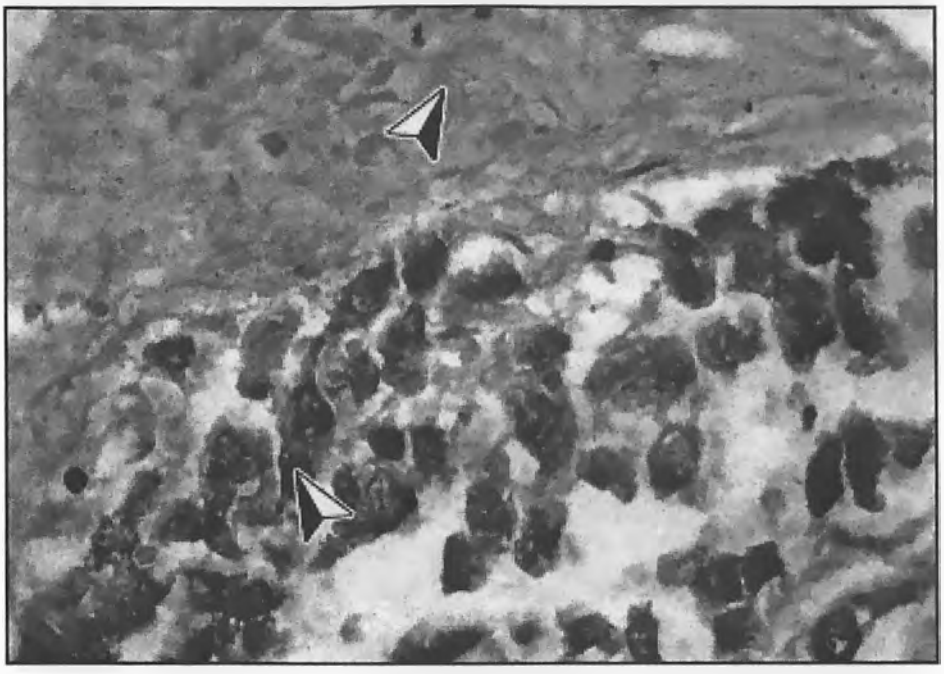

Fig. 3. Testicular biopsy. Seminoma. Places with increasing growth of tumor cells (neighbouring section). Immunoexpression of ANP in the cytoplasm of tumor cells. Intensive collagenation (upper arrow) Monoclonal anti-ANP antibody (Microphoto × 400)

established and possitive ANP immunostaining, as well. (Fig.4, 5). Tumor cells were big, large with centrally located nuclei. In their cytoplasm strong immunoexpression for ANP was observed (Fig. 6, 7, 8).

Discussion

Until now data are lacking for the immunoexpression of ANP under pathological processes as degeneration and tumorogenesis. Examinations of S u e n o b u [8] demonstrated the participation of ANP in endothelial apoptosis. Our findings about intensive immunoexpression of ANP in tumor cells provided support for their hypothesis. Strong immune reaction we found in the cytoplasm of germ cells suggested a possible role of ANP in processes of germ cell differentiation [3]. The presence of natriuretic peptide in the cytoplasm of Sertoli cells supposed that ANP is involved in paracrine control of spermatogenesis.

The increase of connective tissue was a result of pathological process and in the current study we observed places with increasing collagenation, indicative for disturbed nutrition and capillary transport. We found places where seminiferous tubules have saved structure, still not affected by tumor process and there the immune reaction for ANP was strong. The Leydig cells looked small in number, or they are missing and strong immunoreactivity in their cytoplasm can be seen that implied possible local production of ANP in the testis. According to M ü l l e r and M i d d e n d o r f f [5] the basic place of expression of ANP-receptor are germ cells and our data revealing ANP immunoexpression in Leydig and Sertoli cells suggest that ANP is involved in paracrine interac-

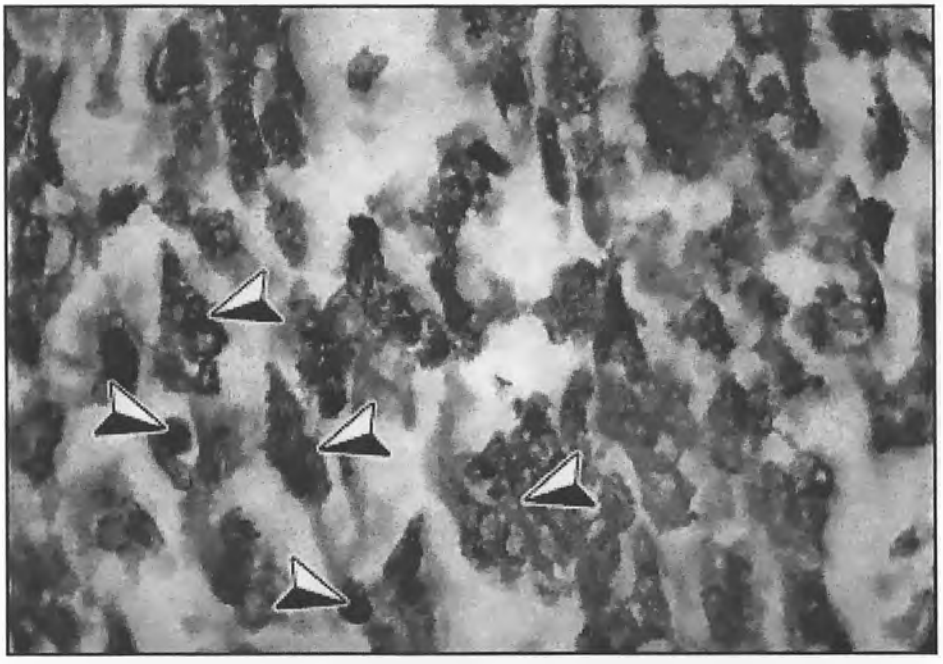

Fig. 4. Testicular biopsy. Seminoma. Tumor cells form nests , between them fine layers with lymphocytes are observed (left arrow). Immunoexpression of ANP in their cytoplasm. Monoclonal anti-ANP antibody (Microphoto × 400)

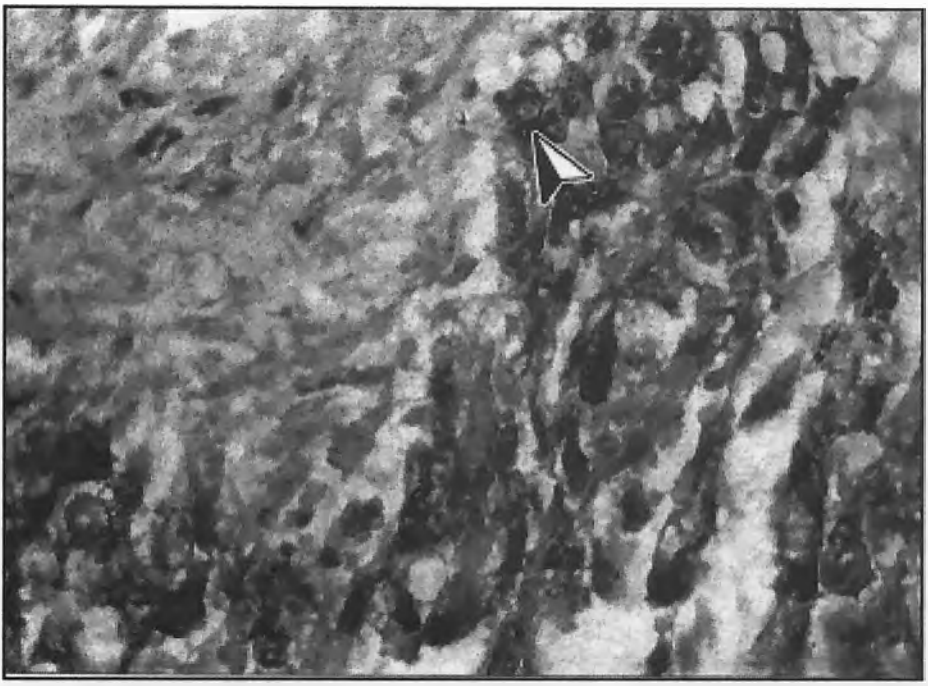

Fig. 5. Testicular biopsy. Seminoma. Nests with tumor cells (neighbouring section). Increasing connective tissue. Immunoexpression of ANP in the cytoplasma of tumor cells. Monoclonal anti-ANP antibody. (Microphoto × 400)

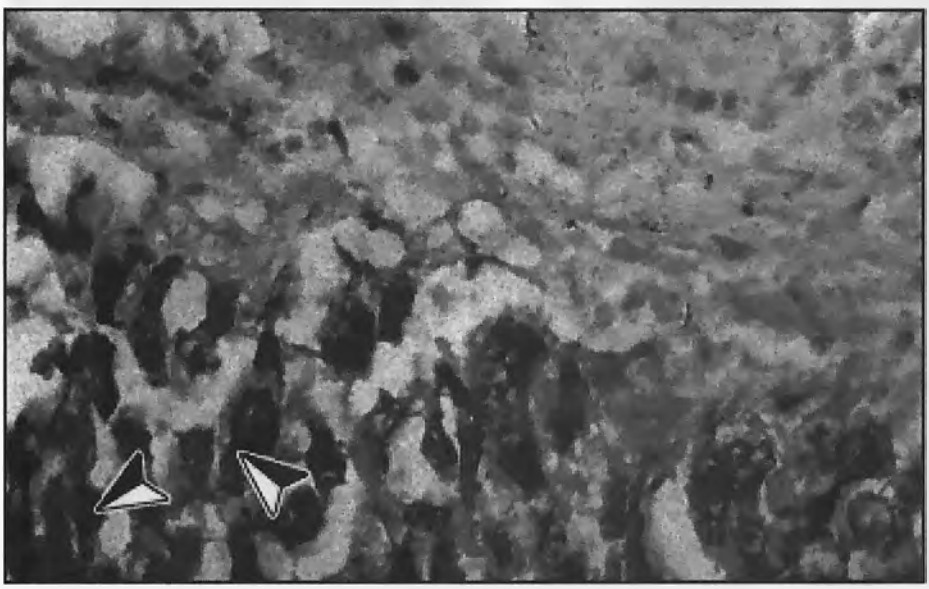

Fig. 6. Testicular biopsy. Seminoma.Big tumor cell with central placed nucleus. In the cytoplasm immunoexpression of ANP is clearly observed. Lymphocyte (left arrow). Monoclonal anti-ANP antibody (Microphoto × 400)

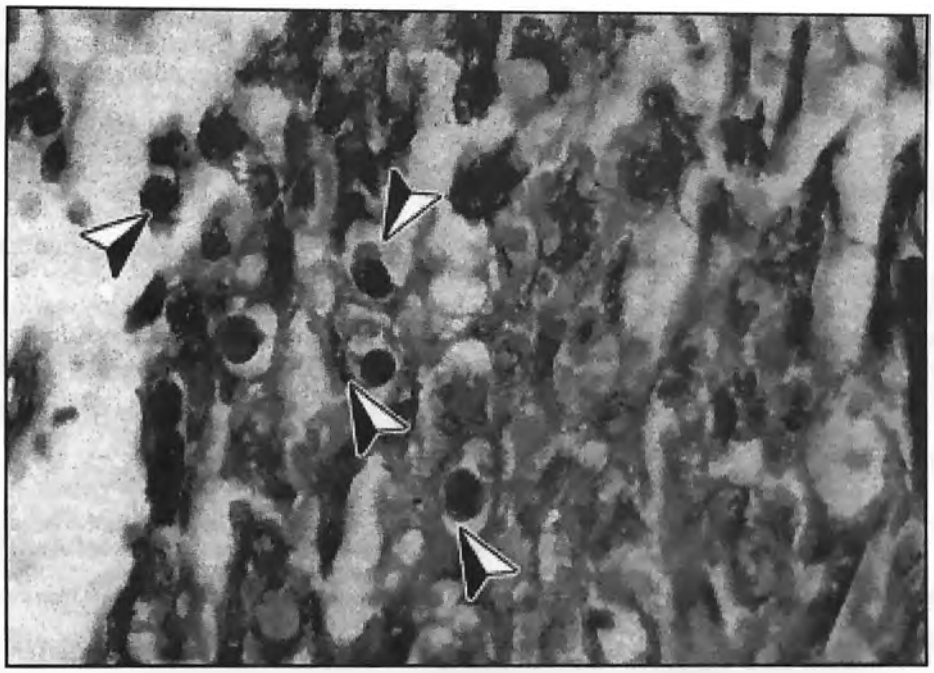

Fig. 7. Testicular biopsy. Seminoma. Big, large tumor cells with immunoexpression of ANP in their cytoplasm, lympfocyte (upper left arrow). Monoclonal anti-ANP antibody (Microphoto × 400)

Fig. 8. Testicular biopsy. Seminoma. Tumor cells with immunoexpression of ANP in their cytoplasm, lymphocyte (lower right arrow). Monoclonal anti-ANP antibody (Microphoto × 400)

tions between germ and somatic cells. Expanding tumor cells formed nests, and around them fine layers with lymphocytes were observed. Tumor cells looked big, large with centrally located nuclei. In patient with seminoma, we found strongest immunoexpression of ANP in the cytoplasm of tumor cells.

Conclusions

1. It could be considered a possible role of ANP in the processes of degeneration and tumorogenesis.
2. The exploring model shows the ability for local production of ANP in the testis which does not depend on degeneration of seminiferous tubules during tumor process, and low level of testosterone.

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