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NEUROTROPHIC FACTORS IN THE TESTIS

Dieter Mutter[1], Ralf Middendorff, and Michail S. Davidoff
'Institute for Hormone and Fertility Research, and Institute of Anatomy, University of Hamburg, Hamburg, Germany

Neurotrophic factors, interacting with neurons to affect their growth, are a subset of the polypeptide growth factors. There is increasing evidence for a broader physiological role of these factors which includes effects on a variety of nonneuronal tissues. Among the cell systems, where neurotrophic factors have been hypothesized to exert local nonneurotrophic activities, the testis is of particular interest. This organ represents a complex biological unit which requires the concerted action of very diverse cell types interacting with each other in order to ensure correct spermatogenesis. As signaling molecules that may be involved in these intercellular communication events, various neurotrophic factors have attained considerable scientific attention. This article intends to summarize the presently available data on the distribution and possible local activities of neurotrophic factors and their receptors in testicular cells and provides further information on local expression sites of some of these factors in the human testis. ***Biomed Rev 1999; 10: 25-30.***

INTRODUCTION

Nerve growth factor (NGF) is the first identified and most intensely studied neurotrophic factor. In addition to its well-established role for the development and survival of neurons in the central and peripheral nervous system, NGF also has a variety of nonneurotrophic activities (1). Target-derived NGF initiates these responses by binding to two distinct cell surface receptors: the tyrosine kinase A (TrkA) receptor and the p75 neurotrophin receptor ($p75^{NTR}$) (reviewed in 2). Based on their close structural and functional relationship, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4/5, and NT-6 have been embodied with NGF to constitute the neurotrophin gene family (2). As does NGF itself, the other neurotrophins bind to $p75^{NTR}$ with a relatively low affinity and interact, *via* high-affinity binding, with a second membrane protein which belongs to the Trk family (1,2). TrkB is regarded as a common receptor for BDNF and NT-4/5, while TrkC is thought to act as a specific receptor for NT-3.

Among the tissues, where neurotrophic factors have been hypothesized to exert local nonneurotrophic activities, the testis is of particular interest. This article highlights the presently available data on the distribution and possible local functions of neurotrophic factors and their receptors in testicular cells. And provides further information on local expression sites of some of these factors in the human testis.

NERVE GROWTH FACTOR IN THE TESTIS

In testis, NGF protein and mRNA levels do not correlate with the innervation by NGF-sensitive nerve fibres, suggesting additional functions within this reproductive organ (3-5). Testicular expression of the NGF gene, detectable as transcript species of 1.3 and/or 1.5 kb by RNA blot analyses, could be demonstrated at first in the testis of rat and mouse (3). In the same study, $mRNA^{NGF}$ was localized to spermatocytes and early spermatids

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Correspondence and reprint requests to Dr Michail S. Davidoff, Institute of Anatomy, University of Hamburg, MartinistraBe 52, D-20246 Hamburg, Germany. E-mail: davidoff@uke.uni-hamburg.de

of adult mouse, through *in situ* hybridization. Findings of NGF-like immunoreactivity in germ cells of rat and mouse testis raised the idea of a nonneurotrophic role for NGF (3,6), possibly related to sperm maturation ormotility (3). Further studies identified the Sertoli cells as local gene expression sites of the $p75^{NTR}$ receptor (7,8). Based on the observed increase in $mRNA^{p75NTR}$ levels after either hypophysectomy, destruction of Leydig cells or by blocking the androgen receptor, and deduced from the ability to suppress these effects by treatments with testosterone or chorionic gonadotropin, the authors concluded that male germ cell-derived NGF may function on Sertoli cells in an androgen-modulated fashion (7). An analysis of the expression of NGF and $p75^{NTR}$ during the spermatogenic cycle revealed $mRNA^{NGF}$ and protein at all stages of the cycle, whereas transcripts for $p75^{NTR}$ were found only in stages VII and VIII, the sites of onset of meiosis(S).

By immunoblot analyses of protein preparations from rat round spermatids,Chene?a/(9) identified two presumable NGF precursors of 31 and 22 kD, whereas the 13 kD mature form of NGF was not observed. Protein extracts from round spermatids were able to extend the viability of Sertoli cells. The elimination of this activity by immunoprecipitation with anti-NGF antibodies suggests that the NGF precursors are responsible for the maintenance of Sertoli cell viability. The specificity of this effect was demonstrated by control treatments, revealing that exogenous NGF but not BDNF or NT-3 and -4/5 was able to rescue Sertoli cells. Consistently, NGF-like andproNGF-like immunoreactivities could be localized to germ cells of the mouse testis (10). And studies performed with isolated and cultured tubules from human testis reveal that NGF affects the morphology and function of Sertoli cells (11). During an investigation designed to elucidate potential regulators of interleukin (IL) production by Sertoli cells, Stephan *et al* (12) demonstrate that NGF stimulates EL-6 levels in a dose-dependent manner but has no effect on IL-1. An additional specific local effect of NGF has been reported by Lonnerberg *et al* (13). These later authors showed a two-fold increase in androgen-binding protein gene expression after infusion of NGF into the rat testis, whereas the mRNA levels of another Sertoli cell protein, urokinase-type plasminogen activator, remained unchanged. Sertoli cell transferrin secretion, used as a parameter to evaluate effects of peritubular myoid cells derived from immature rat testis on Leydig cell functions, likewise was not affected by exogenously added NGF (14).

Several studies have focussed on the developmental regulation of the expression of NGF and its receptors in the embryonic and postnatal testis. Gene expression for $p75^{NTR}$ was found before the onset of spermatogenesis in both mouse and rat testis, and by *in situ* hybridization, the presence of $mRNA^{p75NTR}$ could be demonstrated in the peritubular cells of the embryonic mouse testis (15). Immunohistochemical analyses in the same study showed $p75^{NJR}$-expressing cells to be scattered in the intertubular compartment in the embryonic testis, and to become organized in a cellular layer that surrounds myoid cells of the seminiferous tubules during postnatal development. In the fetal mouse testis at 12.5 days postcoitum, strong $p75^{NTR}$ immunoreactivity was localized to the entire population of mesenchymal cells spread through the interstitial tissue outlining the developing testis cords (16). A careful study of rat embryonic tissues by Wheeler and Bothwell (5) demonstrated $mRNA^{p75NTR}$ in the mesenchymal cells surrounding the epithelia of the developing seminiferous tubules. The initially observed coexpression of neuronal cell adhesion molecule (NCAM), which may serve as an indicator for nerve fiber location, commenced in late stages of morphogenesis, suggesting that $p75^{NTR}$ and NCAM have distinct, early and late functional roles in the development of these epithelial structures.

Two investigations (17,18) of the postnatal developmental expression of NGF receptors in rat testis revealed highest gene expression levels for both $p75^{NTR}$ and TrkA during stages of the functional maturation of this organ. The maximal expression of $mRNA^{p75NTR}$ was detected at day 10(17) orat day 15 (18) stage examined, whereas highest levels of TrkA transcripts were found in 20-day (17) or 24- to 26-day old animals (18). Protein examinations revealed the presence of $p75^{NTR}$ in Sertoli cell membranes by Western blotting (17) and of TrkA by immunohistochemical approaches predominantly in Leydig cells (18). In conjunction with the latter finding, gene transcripts for TrkA could be identified in Leydig cells but not in germ cells of the mouse testis (19). However, the same study failed to detect testicular gene expression for $p75^{NTR}$ during early postnatal development (day 20), but rather assessed highest levels in the adult. Moreover, by analyzing $p75^{NTR}$ gene expression *via* SI nuclease protection assay in different testicular cell types shortly after isolation, specifically-protected transcript fragments are detectable exclusively in spermatocytes and spermatids. Only after several days of culturing, small amounts of $mRNA^{p75NTR}$ are found in Sertoli cells. On the other hand, isolated Leydig cells, peritubular myoid cells and Sertoli cells, but not germ cells, are identified as potential testicular NGF sources. Thus, regarding the topological and/or developmental regulation of $p75^{NTR}$ and NGF gene expression in testis, there is a remarkable discrepancy between this and various other reports. The observed induction of $mRNA^{p75NTR}$ in cultured Sertoli and peritubular myoid cells suggests an autocrine mode of NGF action on these cells.

Potential influences on physiological NGF levels have been proposed in two reports, showing an elevation of NGF content in the testis (and other tissues) in the early period of life in senescence-accelerated mice (20) or an increase of NGF immunoreactivity in the testis and pituitary gland of hypothyroid rats (21), suggesting a possible regulatory link between NGF and thyroid hormone.

While most studies have so far been focussed on the NGF system in rodents, Wrobel *etal* (22) demonstrated recently the expression and developmental regulation of $p75^{NTR}$ in bovine

testis. More importantly, the role of NGF in the human male reproductive system has not yet been characterized systematically. In order to provide initial data on the expression sites of NGF and its receptors in the adult human testis, immunohistochemical analyses have been performed. NGFimmunoreactivity is detectable mainly in Ley dig cells as well as in some Sertoli cells and spermatids of a subset of tubules (Fig. 1), whereas the expression of $p75^{NTR}$ and TrkA is illustrated in Figure 2-4. In addition, reaction products are detectable in the peri tubular myofibroblasts and in a subset of the connective tissue cells of the peritubular tissue. Moderate staining can also be localized to capillary vascular endothelial cells and endothelial and smooth muscle cells of some small- and medium-sized blood vessels.

BRAIN-DERIVED NEUROTROPHIC FACTOR AND NEUROTROPHIN-3, -4/5 IN THE TESTIS

Recently, BDNF gene expression in the human testis is demonstrated by RT-PCR analyses (24), and BDNF protein is detectable in adult Leydig and Sertoli cells by immunocytochemistry (24). An examination of fetal human testis did not reveal any immunoreactivity for BDNF (16). Trace amounts of testicular gene expression of its high-affinity receptor, TrkB, are detected *via* RT-PCR in germ cells but not in Leydig, Sertoli and peritubular myoid cells of the mouse testis (19). In the human testis, there is strong immunoreactivity for TrkB within the cytoplasm of the Leydig cells (Fig. 5). Immunoreactivity of lower staining intensity is detectable also in Sertoli cells and spermatids of some tubules. Functional studies addressing the role of this neurotrophin in the testis have not yet been performed.

In meiotic and early postmeiotic germ cells of the mouse testis, NT-3 has been proposed to be involved in male germ cell development and maturation based on observations of a very strong expression of its receptor TrkC (16). Remarkably, TrkC appears to represent the predominant high-affinity neurotrophin receptor, coexpressed with $p75^{NTR}$ in these cells (19). Presence of transcripts for NT-3 and its receptor in the adult human testis (24) andevidence for both NT-3-like and TrkC-like immunoreactivity in the fetal human testis (16) are reported. The expression of TrkC in the adult human testis is examined by immunohistochemical analyses. As shown in Figure 6, the strongest immunoreactivity is localized to Leydig cells. Reaction products of lower staining intensity are detectable in some peritubular myofibroblasts as well as in some spermatocytes and round spermatids. Although Sertoli cells in general appear unstained, individual cells in certain tubules display distinct immunoreactions (Fig. 6). Direct evidence for functional activities has not yet been provided, but the distribution and the pronounced expression of its components in testis suggest a substantial and specific local role of the NT-3/TrkC system.

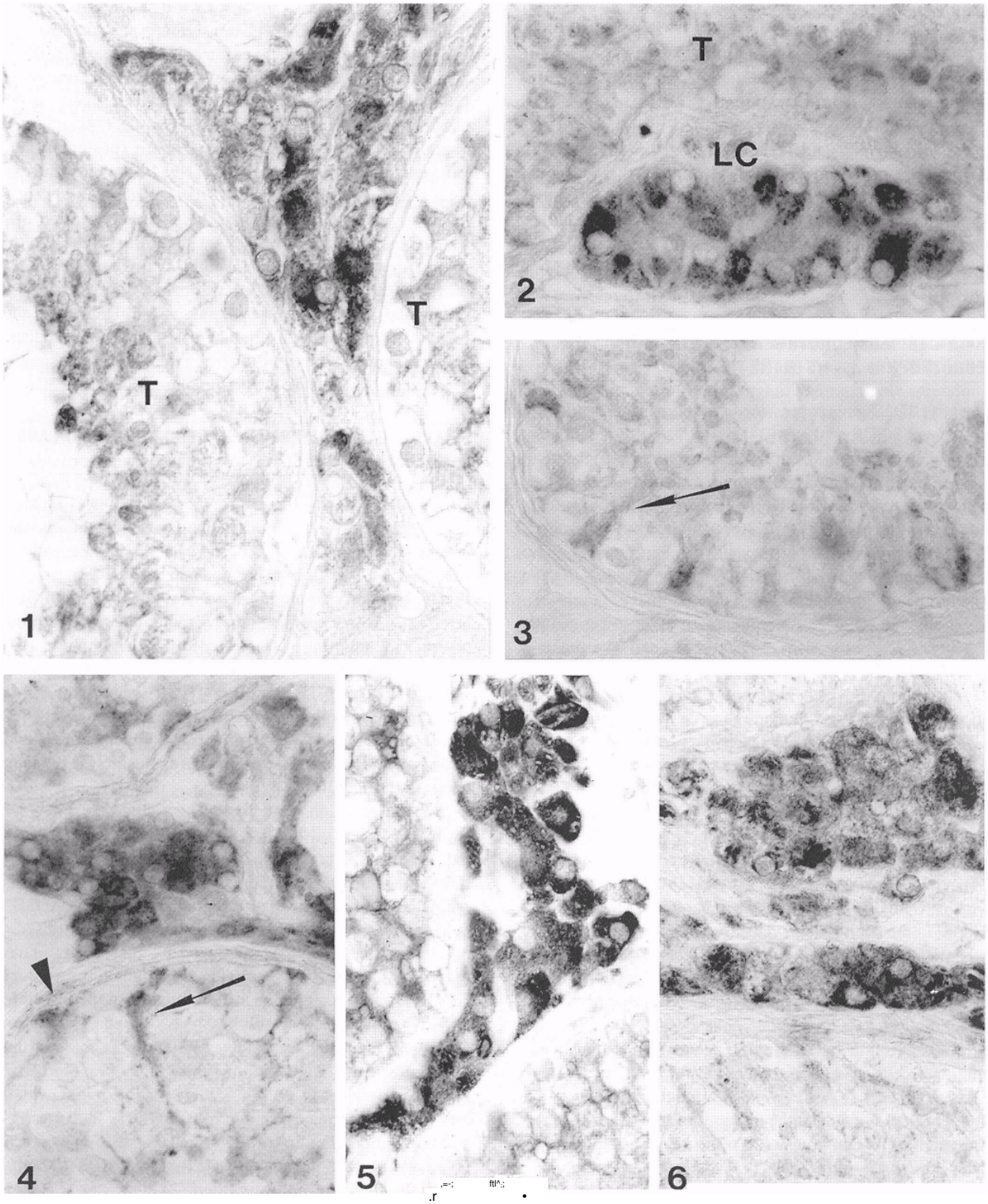
NT-4/5, the latest identified and least understood member of the neurotrophin gene family, interacts with $p75^{NTR}$ and TrkB to exert its biological actions. Several peripheral tissues in human (23) and rat (25) have been shown to express $mRNA^{NT4/5}$. Generally, the level of NT-4/5 transcripts in most tissues is much lower than that of the other neurotrophins. However, $mRNA^{NT\ 4/5}$ is detected at relatively high levels in the premature rat testis (26). By comparison with themRNA expression in various other organs including brain, the highest level of NT-4/5 transcripts overall was found in testes of newborn (postnatal day 1) animals. The recent measurement of NT-4/5 protein in rat tissues by a sensitive immunoassay (27) revealed rarely detectable levels in postnatal tissues with the notable exception of the testis. Consistent with the mRNA data (26), the highest concentration (up to 500 pg/g tissue weight) in this organ is found in the first week after birth. The levels of NT-4/5 decrease during postnatal development but still remain significant in the adult. In conclusion, the high amount of NT-4/5 and its distinct developmental expression pattern in the testis might suggest a function as a growth factor for immature male germ cells.

GLIAL CELL LINE-DERIVED NEUROTROPHIC FACTOR AND NEURTURIN IN THE TESTIS

Glial cell line-derived neurotrophic factor (GDNF), isolated initially from arat glial tumor cell line, is related to the transforming growth fac tor-beta (TGF- (3) superfamily (28). Recently, two other factors, neurturin (NTN) andpersephin (PSP), are identified, sharing a considerable sequence homology with GDNF (29,30). All three factors signal through heteromeric protein complexes consisting of a factor-specific ligand-binding receptor termed GDNF family receptor alpha-1 (GFRoc-1) in case of GDNF, GFRa-2(NTN) orGFRa-4 (PSP), respectively, and the common transmembrane tyrosine kinase receptor *cRet* (31-34). While GDNF and NTN exert effects in both central and peripheral neurons, PSP seems to play arole exclusively in the central nervous system (30).

In the rat testis, $mRNA^{GDNF}$ level is found to be intensively expressed (35,36). Note that during early postnatal development, the gene expression of GDNF in the testis is higher than that in the brain (36). This suggests a pronounced local activity of GDNF in testicular cell differentiation. Evidence for GDNF gene expression in the Sertoli cell line TM 4 (36) raised the possibility that GDNF is produced by these cells *in vivo.* Moreover, *Caoetal* (37) reported that the signalling receptor for GDNF, *cRet,* might function in the regulation of sperm differentiation in mouse, since the highest levels of expression in adult animals arefoundin spermatocytes. Furthermore, marked stimulatory effects of GDNF on the proliferation of rat Sertoli cells have been described (38). Thus, there is some initial evidence for a possible nonneurotrophic role of GDNF in the testis. In addition, gene expression of GDNF and GDNF immunoreactivity as well as the presence of GFRa-1 and GFRa-2 in the human testis are recently reported (24).

Only one published study so far provides information on the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expression and possible role(s) of NTN in testis. Xian *et al* (39) investigated the distribution pattern of $mRNA^{NTN}$ and peptide in several peripheral organs of adult rats. In addition to the pituitary, intestine, and salivary glands, the testis was found to belong the cell systems with highest levels examined, suggesting activities of NTN on nonneuronal cells within this organ.

CONCLUSION

There is a bulk of evidence for physiological functions of neurotrophic factors outside the nervous system. An increasing number of studies suggest that the testis belongs to the peripheral organs where local non-neurotrophic activities play a particular role. In this complex cell system, various neurotrophic factors appear to be involved specifically in the regulation of early developmental processes and in the maintenance of intact spermatogenesis. The preferential expression of many neurotrophic factors and their receptors in human Leydig cells might reflect the neuroendocrine properties of this cell type (40,41).

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REFERENCES

1. Levi-Montalcini R. The saga of the nerve growth factor. *NeuroReport* 1998; 9: R71-R83.
2. Nagtegaal ID, Lakke EAJF, Marani E. Trophic and tropic factors in the development of the central nervous system. *Arch Physiol Biochem* 1998; 106: 161-202.
3. Ayer-LeLievre C, Olson L, Ebendal T, Hallbook F, Persson H. Nerve growth factor mRNA and protein in the testis and epididymis of mouse and rat. *Proc Natl AcadSci USA* 1988; 85: 2628-2632.
4. Seidl K, Holstein AF. Evidence for the presence of nerve growth factor (NGF) and NGF receptors in human testis. *Cell Tissue Res* 1990; 261: 549-554.
5. Wheel erEF, Bos well M. Spatiotemporal patterns of expression of NGF and the low-affinity NGF receptor in rat embryos suggest functional roles in tissue morphogenesis and myogenesis.JTVeMro.s'C! 1992; 12:930-945.
6. Olson L, Ayer-LeLievre C, Ebendal T, Seiger A. Nerve growth factor-like immunoreactivities in rodent salivary glands and testis. *Cell Tissue Res* 1987; 248:275-286.
7. Persson H, Ayer-LeLievre C, Soder O, Villar MJ, Metsis M, Olson L *et al.* Expression of beta-nerve growth factor receptor mRNA in Sertoli cells downregulated by testosterone. *Science* 1990; 247:704-707.
8. Parvinen M, Pelto-Huikko M, Soder O, Schultz R, Kaipia A, Mali P *et al.* Expression of beta-nerve growth factor and its receptor in rat seminiferous epithelium: specific function at the onset *ofmeiosis.JCellBiol* 1992; 117:629-641.
9. Chen Y, Dicou E, Djakiew D. Characterization of nerve growth factor precursor protein expression in rat round spermatids and the trophic effects of nerve growth factor in the maintenance of Sertoli cell viability. *Mol Cell Endocrinol* 1997; 127:129-136.
10. MacGrogan D, Despres G, Rommand R, Dicou E. Expression of the beta-nerve growth factor gene in male sex organs of the mouse, rat, and guinea pig. *J Neurosci Res* 1991; 28:567-573.
11. Seidl K, Holstein AF. Organ culture of human seminiferous tubules: an useful tool to study the role of nerve growth factor in the testis. *Cell Tissue Res* 1990; 261: 539-547.
12. Stephan JP, Syed V, Jegou B. Regulation of Sertoli cell IL-1 and IL-6 production in vitro. *Mol Cell Endocrinol* 1997; 134:109-118.
13. Lonnerberg P, Soder O, Parvinen M, Ritzen EM, Persson H. Beta-nerve growth factor influences the expression of androgen-binding protein messenger ribonucleic acid in the rat testis. *BiolReprodl992;* 47:381-388.
14. Hoeben E, Deboel L, Rombauts L, Heyns W, Verhoeven G. Different cells and cell lines produce factors that modulate Sertoli cell function. *Mol Cell Endocrinol* 1994; 101: 263-275.
15. Russo MA, Odorisio T, Fradeani A, Rienzi L, De Felici M, Cattaneo *Aetal.* Low-affinity nerve growth factor receptor

Figure 1-6. Selected areas of histological sections of human testis. Immunohistochemical analyses have been performed as described previously (40). Magnification for Fig. 1-3: x *158, Fig. 4-6:* x 737. *Figure 1. NGF-beta immunoreactivity. Moderate staining on paraffin sections is localized to Leydig cells. In few tubules (T), also Sertoli cells and some spermatids are positive. Figure 2. Immunoreactivity for* $p75^{NTR}$ *is detectable primarily in the Leydig cells (LC). Figure 3. Within few tubules (T), certain Sertoli cells exhibit marked immunostaining (arrow) for* $p75^{NTR}$*. Figure 4. TrkA immunreactivity in the Leydig cells and Sertoli cells (arrow). A lower staining intensity is visible in the peritubular myofibroblasts (arrowhead) and in some spermatids at the apical portions of the germ epithelium.Figure 5. TrkB immunoreactivity is present within the cytoplasm of Leydig cells and detectable in some Sertoli cells of few tubules. Figure 6. TrkC immunoreactivity, located within the Leydig cells.*

is expressed during testicular morphogenesis and in germ cells at specific stages of spermatogenesis. *Mol ReprodDev* 1994; *3:* 157-166.

16. Russo MA, Giustizieri ML, Favale A, Fantini MC, Campagnolo M, Konda D *et al.* Spatiotemporal patterns of expression of neurotrophins and neurotrophin receptors in mice suggest functional roles in testicular and epididymal morphogenesis. *BiolReprod* 1999 ; 61: 1123-1132.
17. Djakiew D, Pflug B, Dionne C, OnodaM. Postnatal expression of nerve growth factor receptors in the rat testis. *Biol Repwdl994;5l:2l4-22l.*
18. Muller D, Paust HJ, Middendorff R, Davidoff MS. Nerve growth factor (NGF) receptors in male reproductive organs. In: I veil R, Holstein AF, editors. *The Fate of the Male Germ Cell.* Plenum Press, New York, 1997;pp 157-158.
19. Seidl K, Buchberger A, Erck C. Expression of nerve growth factor and neurotrophin receptors in testicular cells suggest novel roles for neurotrophins outside the nervous system. *ReprodFertilDev* 1996; 8:1075-1087.
20. Katoh-Semba R, Semba R, Kashiwamata S, Kato K. An acceleration of age-related increases in levels of the beta-subunit of nerve growth factor in selected tissues from senescence-accelerated mice (SAM-P/8). *JMolNeurosci* 1993; 59:163-175.
21. Calza L, Giardino L, Aloe L. NGF content and expression in the rat pituitary gland and regulation by thyroid hormone. *Brain Res Mol Brain Res* 1997; 51:60-68.
22. Wrobel KH, Bickel D, Schimmel M, Kujat R. Immunohistochemical demonstration of nerve growth factor receptor in bovine testis. *CellTissue Res* 1996;285:189-197.
23. IpNY, Ibanez CF, Nye SH, McClain J, Jones PF, Gies DR *et al.* Mammalian neurotrophin-4: structure, chromosal localization, tissue distribution, and receptor specificity. *Proc NatlAcadSci USA* 1992; 89:3060-3064.
24. Davidoff MS, Muller D, Middendorff R, Koeva Y, Pusch W, Paust HJ *etal.* Neurotrophic factors in developing and adult human testis. *ItalJ Anat Embryol* 1999; 104(Suppl 1): 138.
25. Funakoshi H, Frisen J, Barbany G, TimmuskT, Zachrisson O, Verge *VMKetal.* Differential expression of mRNAsfor neurotrophins and their receptors after axotomy of the sciatic *nerve. J Cell Biol* 1993; 123; 455-465.
26. TimmuskT, Belluardo N, Metsis M, Persson H. Widespread and developmentallly regulated expression of neurotrophin-4 mRNA in rat brain and peripheral tissues. *EurJNeurosci* 1993;5:605-613.
27. Zhang SH, Zhou XF, Deng YS, Rush RA. Measurement of neurotrophin 4/5 in rat tissues by a sensitive immunoassay. *J Neurosci Methods* 1999; 89: 69-74.
28. Lin LF, Doherty D, Lile J, Bektesh S, Collins F. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 1993; 260: 1130-1132.
29. Kotzbauer PT, Lampe PL, Heuckeroth RO, Golden JP, Creedon DJ, Johnson EM *et al.* Neurturin, a relative of glial cell line-derived neurotrophic factor. *Nature* 1996; 467-470.
30. Milbrandt J, Desauvage FJ, Fahrner TJ, BalohRH, Leitner MT, Tansey MG *et al.* Persephin, a novel neurotrophic factorrelated to GDNF and *neurturin. Neuron* 1998; 245-253.
31. Baloh R, Tansey M, Golden J, Creedon D, Heuckeroth R, Keck C *etal.* TrnR2, anovel receptor that mediates neurturin and GDNF signaling through Ret. *Neuron* 1997; 18:793-802.
32. Buj-Bello A, Adu J, Pinon L, Horton A, Thompson J, Rosenthal A *et al.* Neurturin responsiveness requires a GPI-linked receptor and the Ret receptor tyrosine kinase. *Nature* 1997; 387: 721-724.
33. Enokido Y, Desauvage F, Kongo JA, Ninkina N, Rosenthal A, Buchman VL, Davies AM. GFR-a-4 and the tyrosine kinase ret form a functional receptor complex for persephin. Cw/r£w/1998;8:1019-1022.
34. JingSQ,YuYB,FangM,HuZ,HolstPL,BooneTefa/.GFR-oc-2 and GFR-a-3 are two new receptors for ligands of the *DUNFfamily.JBiolChem* 1997;272:33111-33117.
35. Trupp M, Ryden M, Jornvall H, Funakoshi H, Timmusk T, Arenas E *et al.* Peripheral expression and biological activities of GDNF, a new neurotrophic factor for avian and mammalian peripheral neurons. *JCellBiol* 1995; 130:137-148.
36. Choi-Lundberg DL, B ohn MC. Ontogeny and distribution of glial cell line-derived neurotrophic factor (GDNF) mRNA in rat.*DevBrainRes* 1995; 85: 80-88.
37. Cao T, Shannon M, Handel MA, Etkin LD. Mouse ret finger protein (rft) proto-oncogene is expressed at specific stages of mouse spermatogenesis. *Dev Genet* 1996; 19: 309-320
38. Hu J, Shima H, Nakagawa H. Glial cell line-derived neurotrophic factor stimulates Sertoli cell proliferation in the early postnatal period of rat testis development. *Endocrinology* 140:3416-3421.
39. Xian CJ, Huang BR, Zhou XF. Distribution of neurturin mRNA and immunoreactivity in the peripheral tissues of adult rats. Brain/to 1999; 835:247-258.
40. Davidoff M, Schulze W, Middendorff R, Holstein AF. The Leydig cell of the human testis - a new member of the neuroendocrinesystem. *CellTissue Res \993;211:429-439.*
41. Davidoff MS, Middendorff R, Holstein AF. Dual nature of the human Leydig cells. *BiomedRev* 1996; 6: 11-41.