The potential roles of neurotrophins in male reproduction

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Abstract

Neurotrophins are a family of polypeptide growth factors that are required for the proliferation, differentiation, survival, and death of neuronal cells. A growing body of evidence suggests that they may have broader physiological roles in various non-neuronal tissues. The testes are complex non-neuronal organs in which diverse cell types interact to achieve correct spermatogenesis. Both neurotrophins and their receptors have been detected in various cell types from mammalian testes, suggesting that neurotrophins may regulate or mediate intercellular communication within this organ. This review summarizes the existing data on the cellular distribution and possible biological roles of neurotrophins in the testes. The data reported in the literature indicate that neurotrophins affect somatic cell growth and spermatogenesis and imply that they play a role in regulating testicular development and male reproduction.

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Introduction

Two of the most important functions of the mammalian testes are sperm production and testosterone synthesis. The development of the testes and their germ cells is governed by a complex network of cellular processes. The Leydig and Sertoli cells are two classes of somatic cells that play major roles in testicular development and spermatogenesis, and their functions are regulated by FSH and LH in a classic endocrine feedback loop (Amory & Bremner 2001). In addition to the critical roles of hormones in testicular development, factors produced by the Leydig cells, the Sertoli cells and the germ cells are also important. All the known neurotrophins and their receptors have been shown to be expressed in the testis. Nerve growth factor (NGF), a member of the neurotrophin protein family, is produced by the germ cells and acts on the Sertoli cells as a paracrine factor in the postnatal testis (Perrard et al. 2007). Similarly, neurotrophin 3 (NTF3 (NT3)) is expressed by Sertoli cells and promotes the migration of the mesonephros cells and the formation of the seminiferous cord in fetal life (Cupp et al. 2000). These findings suggest that neurotrophins play critical roles in testicular development, acting as both autocrine and paracrine factors. This review discusses the roles of neurotrophins in testicular development.

Neurotrophins and their receptors

Neurotrophins are the best known growth factors in the nervous system. They regulate many aspects of neuronal development and function, including survival, proliferation, differentiation, myelination, apoptosis, axonal growth and synaptic plasticity. The first neurotrophin to be discovered was NGF, which was identified as an essential factor for the survival of motor and sensory neurons 50 years ago (Levi-Montalcini 1987). The second neurotrophin to be identified was brain-derived neurotrophic factor (BDNF), which was initially purified from pig brains and shown to promote neuron survival (Barde et al. 1982). The other known neurotrophins, NTF3 and NT4 (NTF5), are widely expressed in the CNS and are also required for the proper functioning of many neuron types (Hallböök et al. 1991). All neurotrophins are initially synthesized as inactive precursors or pro-neurotrophins that are then processed by proteolysis to form the mature proteins (Hempstead 2002). The mature neurotrophin proteins form stable non-covalent dimers and initiate their biological actions by binding to one of two different classes of receptors: a high-affinity transmembrane tyrosine kinase receptor, Trk, and p75 neurotrophin receptor (p75NTR; Patapoutian & Reichardt 2001), a member of the tumor necrosis factor (TNF) receptor superfamily (Chao 2003). Three members of the TRK receptor family are known in mammals: TRKA, which binds NGF; TRKB, which binds BDNF and NT4; and TRKC, which binds NT3 (Patapoutian & Reichardt 2001). All the four neurotrophins primarily interact with these receptors via their membrane-proximal immunoglobulin-like domains. The p75NTR was originally identified as a low-affinity receptor for NGF.
alone but was subsequently shown to have a similar affinity for other neurotrophins (Rodriguez-Tebar et al. 1990; Fig. 1).

While it was once believed that neurotrophins are essential for the differentiation and survival of various neuronal populations in the CNS and peripheral nervous system, the identification of neurotrophins and their receptors in other organs has prompted suggestions that they may be important in the development and functioning of non-neuronal tissues (Tessarollo 1998). Immunocytochemical data and other evidence suggest that both neurotrophins and their receptors are expressed by a wide range of lung-tissue cells, which implies that they may play significant roles in controlling the structure and function of the lungs (Ricci et al. 2007). Knockout (KO) mouse models showed that Bdnf, Ntf3, TrkB, and TrkC are important in cardiac morphogenesis (Donovan et al. 1996, Wagner et al. 2005). Moreover, there is experimental data showing that NGF is an important factor in muscle regeneration: a phenotypic KO of NGF in adult mice caused severe spleen damage and skeletal muscle atrophy and dystrophy (Ruberti et al. 2000). In females, BDNF was found to regulate oocyte maturation and early embryo development (Kawamura et al. 2005, Yi et al. 2008).

The roles of neurotrophins in testicular development

The first evidence that neurotrophins might be important in testicular development came from the finding that developing testes contain all of the known neurotrophins and their receptors. Subsequent experimental results have clearly shown that the neurotrophins and their receptors are expressed in fetal and adult testicles and in both rodents and humans (Djakiew et al. 1994, Müller et al. 2006). During testicular development, neurotrophins and their receptors are expressed in the germ cells throughout their development and also in somatic cells (Sertoli and Leydig cells; Persson et al. 1990, Koeva et al. 1999), which provides further support for the hypothesis that the activation of their receptors may be important in testicular development and spermatogenesis (Fig. 2).

The NGF–TRKA signaling module

The presence of Ngf transcripts of 1.3 and/or 1.5 kb in rat and mouse testes has been demonstrated by northern blotting. Ngf mRNA was subsequently localized to the spermatocytes and early spermatids in adult mice (Ayer-LeLievre et al. 1988). NGF was also detected in isolated Leydig cells, peritubular myoid cells, and Sertoli

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**Figure 1** The neurotrophins and their receptors. All neurotrophins are initially synthesized as pro-neurotrophins. The mature neurotrophins are generated through proteolysis of pro-neurotrophins. Both pro-neurotrophins and mature neurotrophins can bind to p75NTR. However, mature neurotrophins exhibit more specific interactions with the three TRK receptors: NGF binds specifically to TRKA, TRKB can be activated by both BDNF and NT4, and NT3 binds to TRKC.
cells after several days of culture, suggesting that these somatic cells may be testicular sources of NGF (Seidl et al. 1996, Jin et al. 2006). The gene for the high-affinity NGF receptor TrkA is strongly expressed in postnatal rat testes, but only in non-germ cells. Immunohistochemical analysis revealed the presence of TRKA in the membranes of Leydig cells (Djakiew et al. 1994), a result that was supported by the subsequent finding that TrkA transcripts were present in Leydig cells but not in the germ cells in mouse testes (Seidl et al. 1996). It has also been demonstrated that the Leydig cells are the primary sites of NGF/TRKA expression in both fetal and adult human testes (Müller et al. 2006), although the Sertoli cells and some cellular elements of the germinative epithelium were also TRKA positive (Koeva et al. 1999).

These findings suggest that NGF may regulate testicular development and spermatogenesis via both autocrine and paracrine pathways.

The effects of the NGF produced by male germ cells may be regulated by some of the hormones that are important in male reproduction. In rats gonadotropin may suppress the expression of the NGF receptor. However, hypophysectomy was shown to increase the abundance of the TrkA mRNA in rat seminiferous tubules (Persson et al. 1990). Treatment with hCG for 12 h increased the abundance of TrkA mRNA in premeiotic rat testicule cells. The RNAse protection assay revealed that TrkA mRNA induction was strongest during stages VII and VIII of the cycle (Schultz et al. 2001). Testosterone was also shown to affect the expression of TrkA. Destruction of the Leydig cells or blocking of the androgen receptor increased the abundance of the TrkA mRNA, while testosterone treatment suppressed TrkA (Persson et al. 1990). This suggests that androgens may regulate NGF/TRKA signaling in the testes. Rats treated with 10 μg/kg 4-methylcatechol (4-MC), which is known to induce NGF synthesis, for ten consecutive days exhibited increased plasma levels of NGF and also significantly increased expression of TRKA along with changes in its distribution. In adult control rats TRKA was expressed in the spermatocytes and spermatids, and immunoreactions to TRKA occurred primarily in the spermatocytes. However, in newborn rats treated with 4-MC a different TRKA immunoreaction pattern was observed (Levanti et al. 2006). These findings suggest that the NGF/Trk system plays a role in the physiology of male reproduction. Direct evidence that the NGF/Trk system affects testicular development was obtained from a TrkA-KO mice model. Testes from TrkA-KO mice contained lower number of seminiferous cords at embryonic day 14 (E14) compared with wild-type controls. Histological analysis of the KOs' testes revealed that their development was delayed (Cupp et al. 2002). The TrkA-KO mice produced fewer germ cells than the wild-type controls before E19. Moreover, by postnatal day 19 (P19) the number of apoptotic cells per seminiferous tubule in the testes of the TrkA-KO mice was ten times greater than that for the wild-type (Cupp et al. 2002). Because homozygous mice lacking the TrkA gene did not survive, androgen treatment may suppress the expression of the TrkA gene. Therefore, it is difficult to investigate the role of the NGF/Trk system in spermatogenesis using KO mouse models. Studies using a model based on an in vitro germ cell culture showed that exposing germ cells to NGF for 5 days increased the number of spermatocytes in the secondary meiotic metaphases and decreased the number of round spermatids (Perrard et al. 2007). Conversely, the number of round spermatids was increased when the NGF/Trk system was blocked with K252a, a Trk-specific kinase inhibitor (Perrard et al. 2007).

During spermatogenesis, the Leydig and Sertoli cells play essential roles in germ cell maturation and sperm production (Cheng & Mruk 2004, Svechnikov et al. 2006). Figure 2 Potential functions of the neurotrophins in the control of testicular development. Neurotrophins and their receptors are localized in a wide range of cell types in the testes. Nerve growth factor (NGF) is produced by the Leydig cells, the Sertoli cells and spermatocytes. Brain-derived neurotrophic factor (BDNF) is secreted by the Leydig and Sertoli cells and neurotrophin 4 (NT4) is produced by the Leydig cells. NT3 is mainly produced by the Sertoli cells. Before birth NGF is important for seminiferous cord formation, germ cell differentiation and Sertoli cell viability. NT3 is involved in regulating seminiferous cord formation and germ cell differentiation and also in male sex determination. After birth BDNF and NT4 are essential for mitochondrial activity and for governing viability and apoptosis in ejaculated sperm. NT4 regulates sperm motility and the acrosome reaction.
The observation of differences in the cellular localization of NGF and its receptor in the testes gave rise to the hypothesis that NGF might regulate germ cell differentiation via paracrine signaling. When rats were treated with NGF for 14 days, the levels of mRNA encoding the Sertoli cell-specific receptor androgen-binding protein (ABP) increased significantly, suggesting that androgen signaling might be regulated by NGF during testicular development (Lönnerberg et al. 1992). In an in vitro culture system, the viability of Sertoli cells was rescued by treatment with 10 ng NGF/ml for 12 days but not by BDNF, NT3 or NT4 (Chen et al. 1997). Functional assays performed with a mouse tumor Leydig cell line revealed that NGF treatment increased cellular steroid production (Müller et al. 2006). In order to clarify the paracrine effects of NGF in spermatogenesis it is necessary to identify other NGF-regulated factors expressed by somatic cells in the testes (Müller et al. 2006). Interestingly, recent studies have shown that NGF and TRKA are also present in the ejaculated sperm (Jin et al. 2010a, Li et al. 2010a, 2010b) and that in sperm samples from oligoasthenozoospermic men the levels of both NGF protein in the seminal plasma and TrkA mRNA in the spermatzoa are unusually low (Li et al. 2010a, 2010b). In bovine spermatozoa NGF immunoreactivity was localized to the heads and tails of the spermatzoa, whereas TRKA immunoreactivity was detected in the acrosomal cap, nucleus and tail regions, and treatment of the sperm with exogenous NGF for 2 h had significant effects on leptin secretion, cell viability and sperm apoptosis (Li et al. 2010a, 2010b). NGF also stimulates sperm motility in a time- and dose-dependent manner in golden hamster spermatzoa in vitro and regulates the acrosome reaction via the MAPK signaling pathway (Jin et al. 2010). Ejaculated mammalian spermatzoa are considered to be highly differentiated terminal cells in which no mRNA transcription or translation occurs. It would therefore be very interesting to identify the sources of NGF and TRKA in ejaculated sperm and to determine their effects on sperm function.

The BDNF/NT4–TRKB signaling module

TRKB is a receptor that mediates both BDNF and NT4 signaling (Reichardt 2006). BDNF, NT4, and TRKB have been detected in human testes, and the BDNF protein is localized in adult Leydig and Sertoli cells (Mutter et al. 1999). While some level of TRKB immunoreactivity has been detected in the Leydig cells, the Sertoli cells and the spermatids of some tubules, strong immunoreactivity is only observed in the cytoplasm of the Leydig cells (Mutter et al. 1999). However, TrkB mRNA was only detected in the germ cells in mice testes (Seidl et al. 1996). The levels of Ntf5 mRNA in some of the peripheral tissues are lower than those of other neurotrophins in both humans and rats (Ip et al. 1992, Funakoshi et al. 1993) but it is relatively abundant in premature rat testes. BDNF is expressed at E14, P0, P5, and P20 in rat Sertoli cells, but NT4 has a different expression pattern. Although NT4 is expressed at E14, P0, P5, and P20, NT4 was exclusively found in germ cells. Interestingly, the TrkB mRNA was not detected at E14 by PCR (Levine et al. 2000). This suggests that BDNF and NT4 may have complementary effects that induce the progression of critical events in testicular development and that the low-affinity receptor p75 may be essential in embryonic testis morphogenesis. There is no direct evidence regarding the functional roles of the BDNF/TRKB system in male reproduction due to drastic cell losses in the peripheral nervous systems of BDNF KO mice, which result in early postnatal death (Snider 1994). Interestingly, mice that are homozygous for the Ntf5 mutation do not exhibit any developmental defects and can be viable and fertile (Liebl et al. 2000). This implies that BDNF may be more important than NT4 for survival in mice or that there may be other pathways that can compensate for the absence of NT4 signaling. Although the viability of Sertoli cells cultured in vitro was not rescued by treatment with exogenous BDNF (Chen et al. 1997), increased TrkB expression in injured rat testes has been demonstrated (Moon et al. 2005). Both the mRNAs encoding BDNF, NT4 and TRKB and the corresponding proteins have been detected in ejaculated bull sperm. The BDNF protein was found in the head, neck and tail of the sperm cells, whereas NT4 was localized in the equatorial segment and the mid-section of the sperma-tzoa and TRKB was strongly localized in the acrosome (Li et al. 2012, Wang et al. 2013).

Insulin and leptin can regulate the metabolism of human ejaculated sperm through autocrine mechanisms (Aquila et al. 2005a, 2005b). Interestingly, BDNF can increase the secretion of insulin and leptin in ejaculated sperm, and treatment with exogenous BDNF for 2 h affects sperm apoptosis and viability. Moreover, both BDNF and NT4 have been shown to regulate mitochondrial activity in sperm (Li et al. 2012, Wang et al. 2013). A clinical study has shown that the levels of BDNF mRNA in the sperm and BDNF protein in the seminal plasma of a group of oligoasthenozoospermic men were lower than those in a fertile group, indicating that the decreases in the abundance of BDNF and its mRNA transcript may be associated with pathogenesis in some types of male infertility (Zheng et al. 2011). It is postulated that the BDNF/NT4–TRKB system may promote the survival of testicular cells (Moon et al. 2005, Li et al. 2012, Wang et al. 2013).

The NT3–TRKC signaling module

During fetal and postnatal development, mice testes exhibit high levels of NT3 and TRKC immunoreactivity (Russo et al. 1999). NT3 is mainly localized in the peritubular mesenchymal cells from 14.5-days post coitum to P20, especially in P1. However, NT3...
immunoreactivity decreased gradually during postnatal development and disappeared in the adult testes, suggesting that NTF3 may have a regulatory role in immature testes (Russo et al. 1999). Both NTF3 and the corresponding high-affinity neurotrophin TRKC receptor were found to be expressed in the E14 rat testis, and immunocytochemical analysis of E14 rat testis demonstrated that NTF3 was localized to the Sertoli cells. However, TRKC was present in individual cells of the interstitium at E16 and in preperitubular cells at E18 (Levine et al. 2000). The expression of Ntf3 and Trkc mRNA has also been observed in the testes of human fetuses between weeks 14 and 19 of gestation, with Ntf3 being primarily expressed in the Sertoli and interstitial cells (Robinson et al. 2003). A functional study on the role of the NT3/TRKC system in testis development was conducted using Ntf3 and Trkc-KO mice. At E19 Trkc-KO mice had lower numbers of germ cells and a reduced seminiferous cord area compared with wild-type controls. In both Ntf3 and Trkc-KO testes the interstitial area was smaller than that in wild-type controls at E13 and the number of seminiferous cords was lower than in the wild-type by E14 (Levine et al. 2000, Cupp et al. 2002). Histological analyses of Trkc-KO testes showed that the development of their seminiferous cords appeared to have been delayed relative to wild-type testes (Cupp et al. 2002). Forty percent of E13 testes treated in vitro with TRKC-IgG exhibited significantly reduced cord formation, but TRKA–IgG and TRKB–IgG had no effect on cord formation (Levine et al. 2000). Increased immunoreactivity toward NTF3 and TRKC was observed in the testes of sexually mature male rats treated with lonidamine, an antispermatogenic drug (Artico et al. 2007). These findings indicate that the NT3/TRKC system may be involved in the survival of germ cells and seminiferous cord formation during testicular development. Interestingly, recent studies have shown that NTF3 may be essential in male sex determination. Elevated NTF3 expression is observed during the development of the seminiferous cords and has been shown to promote mesonephros cell migration into the gonads (Cupp et al. 2003). Treatment with a TRKC receptor tyrphostin inhibitor, AG879, was found to inhibit seminiferous cord formation and mesonephros cell migration and also decreased the expression of Sox9, a downstream sex differentiation gene regulated by SRY (Cupp et al. 2003). The action of NT3 as a chemoattractant for cell migration from the mesonephros into the developing gonad seems to be regulated by SRY via a physical interaction between SRY and the Ntf3 promoter that has been observed both in vitro and in vivo (Clement et al. 2011).

The neurotrophins-p75 signaling module

The p75NTR was the first low-affinity NTR to be identified. It has similar affinities for all the known neurotrophins. Interestingly, p75NTR is also a member of the tumor necrosis receptor superfamily with a cytoplasmic domain that includes a ‘death’ domain (Liepinsh et al. 1997). In situ hybridization experiments demonstrated that in both rat and mouse testes Ngfr (p75) mRNA expression occurs before the initiation of spermatogenesis; in embryonic mice, its expression was localized to the peritubular cells (Russo et al. 1994). Immunohistochemical studies reported in the same paper showed that Ngfr-expressing cells are scattered in the intertubular compartment in the embryonic testes but gradually become organized in a cellular layer that surrounds the myoid cells of the seminiferous tubules during postnatal development (Russo et al. 1994). High levels of NGF induced by 4-MC in plasma significantly increased the expression of NGFR in rat testes (Levanti et al. 2006). In adult rats Ngfr was expressed in the spermatogonia, whereas the NGFR protein was detected in a cellular layer that surrounds the seminiferous tubules in newborn rats (Levanti et al. 2006). During the early stages of testicular and epididymal development, the gonadal ridge in the mesenchyme of mice is NGFR positive. During the later phases of organogenesis NTF3 is also expressed by most of the NGFR-positive interstitial cells of the testes (Russo et al. 1999). In addition, smooth muscle actin is expressed by most of the NT3- and NGFR-positive mesenchymal cells, suggesting that the NT3/p75 system may regulate the differentiation of testicular myoid cells and epididymal smooth muscle cells (Russo et al. 1999). Although strong immunoreactivity toward NGF, NTF3, TRKA and TRKC was observed in injured rat testes following treatment with Lonidamine, the observed levels of NGFR immunoreactivity were lower than those in untreated rats (Artico et al. 2007). Based on these results, one could reasonably suggest that the neurotrophin/p75 system may have important functions in early testicular development. In adult testes p75 may serve to balance the effects of the neurotrophin/p75 system and the TNF/p75 system and thereby control testicular morphology.

Concluding remarks

Neurotrophins and their receptors are widely expressed in the testes, implying that they have functional roles in testicular development. Immunohistochemical studies on the cellular localization of neurotrophins and their receptors have shown that they are present in almost every cell type present within this organ. There is evidence that TRKA activity may be regulated by gonadotropins in the testes, suggesting a link between NGF/TRKA signaling and the gonadotropins. Further investigations focusing on the interactions between the neurotrophins and gonadotropins will be required in order to properly understand the role of neurotrophins in testicular development. Treatment with exogenous NGF causes increases in the expression of mRNAs encoding...
ABPs, implying that NGF may regulate spermatogenesis by mediating the effects of testosterone. In addition to its ability to promote germ cell development in vitro, it has been shown that NGF can regulate sperm motility and apoptosis. At the same time, it also regulates the acrosome reaction via the MAPK signaling pathway, suggesting that NGF/TRKA signaling ties into multiple important processes. Although defects have been observed in the testes of certain neurotrophin KO, such animals are not ideal tools for studying the role of neurotrophins in male reproduction because of their tendency to die shortly after birth due to severe CNS defects. Further studies will be required to determine the exact mechanisms by which neurotrophins affect male reproduction, possibly using conditional KO animal models. In addition, there is evidence of an interaction between SRY and the Ntf3 promoter, which implies that Ntf3 may have some functional redundancy. Overall, the literature data strongly suggest that the neurotrophins have vital but complex roles in the regulation of testicular development.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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