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 testicles 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 (Rodríguez-Tébar *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

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.

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.

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 gonadotropins may suppress the expression of the NGF receptor. However, hypophysectomy was shown to increase the abundance of the *TrkA* mRNA transcript and the number of NGF receptor-positive cells in rat seminiferous tubules (Persson *et al.* 1990). Treatment with hCG for 12 h increased the abundance of *TrkA* mRNA in premeiotic rat testicle 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 spermatozoa 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/TRKA 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 die shortly after birth, it is difficult to investigate the role of the NGF/TRKA system in spermatogenesis using gene 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/TRKA 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.*

2010). 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.* 2010, 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 spermatozoa are unusually low (Li *et al.* 2010a, 2010b). In bovine spermatozoa NGF immunoreactivity was localized to the heads and tails of the spermatozoa, 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 spermatozoa *in vitro* and regulates the acrosome reaction via the MAPK signaling pathway (Jin *et al.* 2010). Ejaculated mammalian spermatozoa 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 spermatozoa 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 NTF3 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, NTF3

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 tyrosine kinase 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 testicles (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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References

- Amory JK & Bremner W 2001 Endocrine regulation of testicular function in men: implications for contraceptive development. *Molecular and Cellular Endocrinology* **182** 175–179. (doi:10.1016/S0303-7207(01)00562-7)
- Aquila S, Gentile M, Middea E, Catalano S & Andò S 2005a Autocrine regulation of insulin secretion in human ejaculated spermatozoa. *Endocrinology* **146** 552–557. (doi:10.1210/en.2004-1252)
- Aquila S, Gentile M, Middea E, Catalano S, Morelli C, Pezzi V & Andò S 2005b Leptin secretion by human ejaculated spermatozoa. *Journal of Clinical Endocrinology and Metabolism* **90** 4753–4761. (doi:10.1210/jc.2004-2233)
- Artico M, Bronzetti E, Saso L, Felici LM, D'Ambrosio A, Forte F, Grande C & Ortolani F 2007 Immunohistochemical profile of some neurotransmitters and neurotrophins in the seminiferous tubules of rats treated by lisdamine. *European Journal of Histochemistry* **51** 19–24.
- Ayer-LeLievre C, Olson L, Ebendal T, Hallböök F & Persson H 1988 Nerve growth factor mRNA and protein in the testis and epididymis of mouse and rat. *PNAS* **85** 2628–2632. (doi:10.1073/pnas.85.8.2628)
- Barde YA, Edgar D & Thoenen H 1982 Purification of a new neurotrophic factor from mammalian brain. *EMBO Journal* **1** 549–553.
- Chao MV 2003 Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nature Reviews. Neuroscience* **4** 299–309. (doi:10.1038/nrn1078)
- Chen Y, Dicou E & Djakiew D 1997 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. *Molecular and Cellular Endocrinology* **127** 129–136. (doi:10.1016/S0303-7207(96)04001-4)
- Cheng CY & Mruk DD 2004 Sertoli–Sertoli and Sertoli–germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocrine Reviews* **25** 747–806. (doi:10.1210/er.2003-0022)
- Clement TM, Bhandari RK, Sadler-Riggleman I & Skinner MK 2011 SRY directly regulates the neurotrophin 3 promoter during male sex determination and testis development in rats. *Biology of Reproduction* **85** 277–284. (doi:10.1095/biolreprod.110.090282)
- Cupp AS, Kim GH & Skinner MK 2000 Expression and action of neurotrophin-3 and nerve growth factor in embryonic and early postnatal rat testis development. *Biology of Reproduction* **63** 1617–1628. (doi:10.1095/biolreprod63.6.1617)
- Cupp AS, Tessarollo L & Skinner MK 2002 Testis developmental phenotypes in neurotrophin receptor *trkA* and *trkC* null mutations: role in formation of seminiferous cords and germ cell survival. *Biology of Reproduction* **66** 1838–1845. (doi:10.1095/biolreprod66.6.1838)
- Cupp AS, Uzumcu M & Skinner MK 2003 Chemotactic role of neurotrophin 3 in the embryonic testis that facilitates male sex determination. *Biology of Reproduction* **68** 2033–2037. (doi:10.1095/biolreprod.102.012617)
- Djakiew D, Pflug B, Dionne C & Onoda M 1994 Postnatal expression of nerve growth factor receptors in the rat testis. *Biology of Reproduction* **51** 214–221. (doi:10.1095/biolreprod51.2.214)
- Donovan MJ, Hahn R, Tessarollo L & Hempstead BL 1996 Identification of an essential nonneuronal function of neurotrophin 3 in mammalian cardiac development. *Nature Genetics* **14** 210–213. (doi:10.1038/ng1096-210)
- Funakoshi H, Frisén J, Barbany G, Timmusk T, Zachrisson O, Verge VM & Persson H 1993 Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *Journal of Cell Biology* **123** 455–465. (doi:10.1083/jcb.123.2.455)
- Hallböök F, Ibáñez C & Persson H 1991 Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in *Xenopus* ovary. *Neuron* **6** 845–858. (doi:10.1016/0896-6273(91)90180-8)
- Hempstead BL 2002 The many faces of p75NTR. *Current Opinion in Neurobiology* **12** 260–267. (doi:10.1016/S0959-4388(02)00321-5)
- Ip NY, Ibáñez CF, Nye SH, McClain J, Jones PF, Gies DR, Belluscio L, Le Beau MM, Espinosa R III, Squinto SP et al. 1992 Mammalian neurotrophin-4: structure, chromosomal localization, tissue distribution, and receptor specificity. *PNAS* **89** 3060–3064. (doi:10.1073/pnas.89.7.3060)
- Jin W, Arai KY, Shimizu K, Kojima C, Itoh M, Watanabe G & Taya K 2006 Cellular localization of NGF and its receptors *trkA* and *p75LNGFR* in male reproductive organs of the Japanese monkey, *Macaca fuscata fuscata*. *Endocrine* **29** 155–160. (doi:10.1385/ENDO:29:1:155)
- Jin W, Tanaka A, Watanabe G, Matsuda H & Taya K 2010 Effect of NGF on the motility and acrosome reaction of golden hamster spermatozoa *in vitro*. *Journal of Reproduction and Development* **56** 437–443. (doi:10.1262/jrd.09-219N)
- Kawamura K, Kawamura N, Mulders SM, Sollewijn Gelpke MD & Hsueh AJW 2005 Ovarian brain-derived neurotrophic factor (BDNF) promotes the development of oocytes into preimplantation embryos. *PNAS* **102** 9206–9211. (doi:10.1073/pnas.0502442102)
- Koeva Y, Davidoff M & Popova L 1999 Immunocytochemical expression of *p75LNGFR* and *trkA* in Leydig cells of the human testis. *Folia Medica* **41** 53–58.
- Levanti MB, Germanà A, de Carlos F, Ciriaco E, Vega JA & Germanà G 2006 Effects of increased nerve growth factor plasma levels on the expression of *TrkA* and *p75* in rat testicles. *Journal of Anatomy* **208** 373–379. (doi:10.1111/j.1469-7580.2006.00528.x)
- Levi-Montalcini R 1987 The nerve growth factor: thirty-five years later. *EMBO Journal* **6** 1145–1154.
- Levine E, Cupp AS & Skinner MK 2000 Role of neurotrophins in rat embryonic testis morphogenesis (cord formation). *Biology of Reproduction* **62** 132–142. (doi:10.1095/biolreprod62.1.132)
- Li C, Zheng L, Wang C & Zhou X 2010a Absence of nerve growth factor and comparison of tyrosine kinase receptor A levels in mature spermatozoa from oligoasthenozoospermic, asthenozoospermic and fertile men. *Clinica Chimica Acta* **411** 1482–1486. (doi:10.1016/j.cca.2010.06.002)

- Li CJ, Sun YF, Yi KL, Ma YH, Sun YL, Zhang W & Zhou X 2010b Detection of nerve growth factor (NGF) and its specific receptor (TrkA) in ejaculated bovine sperm, and the effects of NGF on sperm function. *Theriogenology* **74** 1615–1622. (doi:10.1016/j.theriogenology.2010.06.033)
- Li C, Li C, Zhu X, Wang C, Liu Z, Li W, Lu C & Zhou X 2012 The expression and putative role of brain-derived neurotrophic factor and its receptor in bovine sperm. *Theriogenology* **77** 636–643. (doi:10.1016/j.theriogenology.2011.09.003)
- Liebl DJ, Klesse LJ, Tessarollo L, Wohlman T & Parada LF 2000 Loss of brain-derived neurotrophic factor-dependent neural crest-derived sensory neurons in neurotrophin-4 mutant mice. *PNAS* **97** 2297–2302. (doi:10.1073/pnas.040562597)
- Liepinsh E, Ilag LL, Otting G & Ibáñez CF 1997 NMR structure of the death domain of the p75 neurotrophin receptor. *EMBO Journal* **16** 4999–5005. (doi:10.1093/emboj/16.16.4999)
- Lönnberg P, Söder O, Parvinen M, Ritzén EM & Persson H 1992 β -Nerve growth factor influences the expression of androgen-binding protein messenger ribonucleic acid in the rat testis. *Biology of Reproduction* **47** 381–388. (doi:10.1095/biolreprod47.3.381)
- Moon C, Yasuzumi F, Okura N, Kim H, Ahn M & Shin T 2005 Enhanced expression of tyrosine kinase receptor a in germ cells of rat testis with acute experimental testicular torsion. *Urologia Internationalis* **74** 79–85. (doi:10.1159/000082715)
- Müller D, Davidoff MS, Bargheer O, Paust HJ, Pusch W, Koeva Y, Jezek D, Holstein AF & Middendorff R 2006 The expression of neurotrophins and their receptors in the prenatal and adult human testis: evidence for functions in Leydig cells. *Histochemistry and Cell Biology* **126** 199–211. (doi:10.1007/s00418-006-0155-8)
- Mutter D, Middendorff R & Davidoff MS 1999 Neurotrophic factors in the testis. *Biomedical Reviews* **10** 25–30.
- Patapoutian A & Reichardt LF 2001 Trk receptors: mediators of neurotrophin action. *Current Opinion in Neurobiology* **11** 272–280. (doi:10.1016/S0959-4388(00)00208-7)
- Perrard M, Vigier M, Damestoy A, Chapat C, Silandre D, Rudkin BB & Durand P 2007 β -Nerve growth factor participates in an auto/paracrine pathway of regulation of the meiotic differentiation of rat spermatocytes. *Journal of Cellular Physiology* **210** 51–62. (doi:10.1002/jcp.20805)
- Persson H, Ayer-Le Lievre C, Söder O, Villar MJ, Metsis M, Olson L, Ritzén M & Hökfelt T 1990 Expression of β -nerve growth factor receptor mRNA in Sertoli cells downregulated by testosterone. *Science* **247** 704–707. (doi:10.1126/science.2154035)
- Reichardt LF 2006 Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **361** 1545–1564. (doi:10.1098/rstb.2006.1894)
- Ricci A, Graziano P, Bronzetti E, Saltini C, Sciacchitano S, Cherubini E, Renzoni E, Du Bois RM, Grutters JC & Mariotta S 2007 Increased pulmonary neurotrophin protein expression in idiopathic interstitial pneumonias. *Sarcoidosis, Vasculitis, and Diffuse Lung Diseases* **24** 13–23.
- Robinson LL, Townsend J & Anderson RA 2003 The human fetal testis is a site of expression of neurotrophins and their receptors: regulation of the germ cell and peritubular cell population. *Journal of Clinical Endocrinology and Metabolism* **88** 3943–3951. (doi:10.1210/jc.2003-030196)
- Rodriguez-Tébar A, Dechant G & Barde YA 1990 Binding of brain-derived neurotrophic factor to the nerve growth factor receptor. *Neuron* **4** 487–492. (doi:10.1016/0896-6273(90)90107-Q)
- Ruberti F, Capsoni S, Comparini A, Di Daniel E, Franzot J, Gonfloni S, Rossi G, Berardi N & Cattaneo A 2000 Phenotypic knockout of nerve growth factor in adult transgenic mice reveals severe deficits in basal forebrain cholinergic neurons, cell death in the spleen, and skeletal muscle dystrophy. *Journal of Neuroscience* **20** 2589–2601.
- Russo MA, Odorisio T, Fradeani A, Rienzi L, De Felici M, Cattaneo A & Siracusa G 1994 Low-affinity nerve growth factor receptor is expressed during testicular morphogenesis and in germ cells at specific stages of spermatogenesis. *Molecular Reproduction and Development* **37** 157–166. (doi:10.1002/mrd.1080370206)
- Russo MA, Giustizieri ML, Favale A, Fantini MC, Campagnolo L, Konda D, Germano F, Farini D, Manna C & Siracusa G 1999 Spatiotemporal patterns of expression of neurotrophins and neurotrophin receptors in mice suggest functional roles in testicular and epididymal morphogenesis. *Biology of Reproduction* **61** 1123–1132. (doi:10.1095/biolreprod61.4.1123)
- Schultz R, Metsis M, Hökfelt T, Parvinen M & Peltö-Huikko M 2001 Expression of neurotrophin receptors in rat testis. Upregulation of TrkA mRNA with hCG treatment. *Molecular and Cellular Endocrinology* **182** 121–127. (doi:10.1016/S0303-7207(01)00551-2)
- Seidl K, Buchberger A & Erck C 1996 Expression of nerve growth factor and neurotrophin receptors in testicular cells suggest novel roles for neurotrophins outside the nervous system. *Reproduction, Fertility, and Development* **8** 1075–1087. (doi:10.1071/RD9961075)
- Snider WD 1994 Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* **77** 627–638. (doi:10.1016/0092-8674(94)90048-5)
- Svechnikov K, Izzo G, Landreh L, Weisser J & Söder O 2010 Endocrine disruptors and Leydig cell function. *Journal of Biomedicine & Biotechnology* **2010** 1–11. (doi:10.1155/2012/684504)
- Tessarollo L 1998 Pleiotropic functions of neurotrophins in development. *Cytokine & Growth Factor Reviews* **9** 125–137. (doi:10.1016/S1359-6101(98)00003-3)
- Wagner N, Wagner KD, Theres H, Englert C, Schedl A & Scholz H 2005 Coronary vessel development requires activation of the TrkB neurotrophin receptor by the Wilms' tumor transcription factor Wt1. *Genes and Development* **19** 2631–2642. (doi:10.1101/gad.346405)
- Wang CQ, Zhang J, Sun YF, Wang D, Li CJ, Li WH, Lu C, Liu Z, Wu H, Hou XF *et al.* 2013 Detection of neurotrophin-4 (NT-4) in ejaculated bull spermatozoa and its effect on spermatozoa mitochondrial activity. *Reproduction in Domestic Animals* **48** 131–136. (doi:10.1111/j.1439-0531.2012.02048.x)
- Yi KL, Zhou X, Shi DS, Chen HH, Qin QL, Chen Y, Li CJ, Zhao ZH & Xing SY 2008 The mRNA expression of brain-derived neurotrophic factor in oocytes and embryos and its effects on the development of early embryos in cattle. *Animal* **2** 1786–1794. (doi:10.1017/S1751731108002838)
- Zheng L, Li C, Sun Y, Liu Z & Zhou X 2011 Expression of brain-derived neurotrophic factor in mature spermatozoa from fertile and infertile men. *Clinica Chimica Acta* **412** 44–47. (doi:10.1016/j.cca.2010.08.045)

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