The Roles of Nerve Growth Factor and Its Receptors in Leydig Cells Development and Function

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Abstract

Many reports have established that the action of Nerve growth factor (NGF) is not restricted to the nervous system but can affect a broad range of non-neuronal cells. There are increasing evidences for a broader physiological role of NGF effects on the testis. Leydig cells is of particular interest. Leydig cells can secrete androgens which is a steroid hormone, synthesized in Leydig cells from cholesterol, the testosterone from Leydig cells, is of the main circulating androgen in the body. Local functions for NGF have been demonstrated in the developing and mature testis of rodents. The testis have the presence of nerve growth factor (NGF), as well as neurotrophin receptors p75NTR, TrkA and immunohistochemical analyses, carried out on prenatal human testis, identified a broad expression of NGF and NGF receptors, predominantly localized to Leydig cells. NGF performs function through NGF/TrkA/p75 system, which main expression sites are in germ cells, sertoli cells and Leydig cells. NGF may be a potential regulator of steroidogenesis in Leydig cell. Moreover, these regulations may involve in several different pathway. Here we present the presently available data on the distribution of possible local activities of NGF and its receptors in Leydig cells, provides further information about effect of NGF on the Leydig cell function and development.

Keywords

Nerve growth factor (NGF); TrkA; p75; Leydig cell; Steroidogenesis; male reproduction.

1. Introduction

Nerve growth factor (NGF), as the first identified neurotrophic protein, was discovered originally in mouse sarcoma 180 [1] and regulates the survival, growth and differentiation of neurons. NGF is known to have important functions in the development and maintenance of sensory and sympathetic neurones in the mammalian nervous system. NGF is clearly the best-characterized neurotrophic protein. It acts on sympathetic and neural crest-derived sensory neurons [2], and is also present in the central nervous system (CNS) where it serves a trophic function in the development and maintenance of basal forebrain cholinergic neurons [3]. Considerable evidence has accumulated over the last decade to indicate that the actions of NGF extend far beyond “classical” effects on cells of the nervous system, to encompass a role for this molecule in the cells of outside the nerve system [4], an candidate is Leydig cells in male reproduction. Leydig cells are outstanding NGF receptor expression sites [5], which show co-expression of p75NTR and TrkA [6]. Moreover, Leydig cells are potential NGF sources in testicular [7]. During Leydig cells development, NGF and its receptors have shown significant changes at mRNA and protein levels, indicating NGF may be an important regulator [8,9,10].

NGF has been proved to exert local nonneurotrophic activities. This article highlights the presently available data on the distribution and possible local functions of NGF and its receptors in Leydig cells, and provides information on their local expression sites. In addition, this article have shown the possible mechanisms that how NGF influences on Leydig cell.

2. NGF and its receptors in Leydig cells

High levels of NGF protein and mRNA did not correlate with the low density of innervations by NGF-sensitive nerve fibres, indicating potential biological roles of NGF and its rectptors in the male reproduction [6,11,12]. Nerve growth factor (NGF), the most intensely studied neurotrophic factor, mediates cellular effect by interactions with two distinct receptor entities, designated as TrkA and p75NTR [13]. Target-derived NGF initiates down-stream responses by binding to the two distinct cell surface receptors. NGF can bind preferentially to TrkA. It can be regulated by receptor dimerization, structural modifications or association with the p75 receptor [14,15]. The p75 receptor can bind to NGF, and also acts as a coreceptor for Trk receptors. Expression of p75 can increase the affinity of TrkA for NGF and can enhance its specificity for NGF [16,17,18]. In the male mouse, the submandibular gland is a major source of NGF [19]. Relatively high levels of NGF protein were also found in the seminal vesicle [20] and prostate [21].Testicular expression of the NGF gene, detectable as transcript species of 1.3 and/or 1.5 kb by RNA blot analyses, and expression of NGF protein could be demonstrated in the testis of rat and mouse [17]. In human testis, by immunohistochemical investigations, NGF was detectable exclusively within the cytoplasm of Leydig cells, whereas other somatic cells and germ cells did not show any significant staining; Leydig cell appear to represent the major sites of TrkA expression and a pronounced staining for p75NTR in the human testis [6]. In the same study, function assays of NGF effects on Leydig cells were performed with immortalized Leydig cells, MA-10(Lack of CYP17 gene expression, the major steroid production from MA-10 cells is progesterone). Treatment for 24h with NGF (10 ng/ml) markedly increased both basal and hCG-stimulated progesterone productions, otherwise, a proliferation assay showed that NGF incubation didn't elicit increases in cell number. This holds for both hCG-stimulated and basal progesterone synthesis, consistent with previous study [7], indicating an effective role in regulation of Leydig cell differentiation. Olson et al have shown that the NGF exists in the spermatogenic cells at all ages, in mature spermatozoon and the seminiferous tubules of rats and mice [22]. Subsequent research has indicated that NGF participates in the adjustment of testosterone. In conjunction with the latter finding, Koeva et al found the expression of p75 and TrkA in human Leydig cells [23]. Leydig cells were one of the potential origins of testicular NGF. NGF and its receptor played a certain role in adjusting the synthesis of steroid hormone in Leydig cells through autocrine and paracrine function. It was postulated that the adjustment of the activity of the steroid gene affects the function of the Leydig cell, otherwise, catecholamines affects testicular steroidogenesis, so testicular NGF may also be involved in the regulation of testicular function through the effects on nerve system [24]. In the fetal mouse testis at 12.5 days postcoitum, strong p75NTR immunoreactivity was localized to the entire population of mesenchymal cells spread through the interstitial tissue outlining the developing testis cords [25]. A careful study of rat embryonic tissues by Wheeler and Bothwell demonstrated mRNAp75NTR in the mesenchymal cells surrounding the epithelia of the developing seminiferous tubules [26].

In fact, as previously mentioned, there has been a bulk of reports, showing that Leydig cells are a potential source of NGF and possessed neuroendocrine properties [7,27,28,29]. NGF exerted effect on Leydig cells in either an autocrine or paracrine manner.

3. NGF and Its receptors During Leydig cells development

There are two different populations of Leydig cells (LCs), namely of the fetal and adult type, can be identified in the testis during development [30,31]. The adult type undergoes four stage model of differentiation [32] and form population of Leydig cell, including Stem Leydig cells (SLCs), Progenitor Leydig cells (PLCs), Immature Leydig cells (ILCs) and Adult Leydig cells (ALCs). The adult-type LCs emerge during pubertal sexual development. The LC maturation undergoes a complex process of proliferation and differentiation under the control of endocrine and paracrine signals [33,34]. In the adult rat, once a critical mass of mature LCs is achieved, the proliferative activity of the LC population becomes less mitotically active such that fully differentiated adult Leydig cells no longer divide. However, once adult male rat was treated with ethylene dimethanesulfonate (EDS), an

alkylating toxicant that is selectively cytotoxic for differentiated Leydig cells, adult Leydig cells are depleted, and the process of Leydig cell development will be reproduced during adult life, as a result, a new generation of Leydig cells is formed [35,36,37,38,39,40,41,42,43,44]. The evidences have indicated that NGF have been involved in Leydig cells development from our study which have been shown that NGF in the rats' testis have increased and reached a peak at 7 days following EDS, then return to the normal level at 28 days. In order to assess the NGF action, an organ culture of isolated seminiferous tubules was used to obtain more information concerning NGF involved in the Leydig cell development, NGF induces to promote development of stem Leydig cell [45]. Furthermore, several published studies have focussed on the regulation of the expression of NGF and its receptors during Leydig cells development. In the fetal mouse testis at 12.5 days postcoitum, strong p75NTR immunoreactivity was localized to the entire population of mesenchymal cells spread through the interstitial tissue outlining the developing testis cords [46]. An attentive study about rat embryonic tissues [26] revealed mRNAp75NTR in the mesenchymal cells surrounding the epithelia of the developing seminiferous tubules. Immunohistochemical analyses showed p75NTR-expressing cells were located in the intertubular compartment in the embryonic testis, and during postnatal development these cells become organized in a cellular layer that surrounds myoid cells of the seminiferous tubules [25]. Mŭller et al divided the fetal testes into three groups: The 1st group comprised testes from fetuses at the 15th and 16th week of gestation; the 2nd group, testes from fetuses between the 17th and 25th week of gestation, while the 3rd group comprised from between the 26th and 34th gestational week. The cytochrome P450 side chain cleavage enzyme (CytP450), representing the rate-limiting enzyme of steroidogenesis, served to a Leydig cell differentiation marker protein [6]. In testes of the 1st group, strong cytochrome P450scc-IR was detectable in Leydig cell and increased in 18-week-old fetuses. Between the 21st and 25th week of gestation, a gradual decrease in the staining intensity and also in the number of positive Leydig cells could be identified. These changes was also detectable in testes of the 3rd group in which the number of positive Leydig cells has been reduced further. The same changes happened to NGF and its receptors. NGF-immunoreactivity (-IR) in testes of the 1st group was specifically localized to Leydig cells. After the 21st week of gestation, we recognized decreases in Leydig cell staining. p75NTR had a low to moderate staining in Leydig cells in the 1st group. In Leydig cells of testes from the 2nd group, an increase happened in the staining intensity. From the 24th week on, a gradual decrease in the staining intensity of p75NTR-IR in Leydig cells. Until the 30th week, Leydig cells with low to moderate immunostaining were detectable, but only single Leydig cells remain positive during the 34th week of gestation. Leydig cells of the 1st group exhibit TrkA-IR. In the 2nd group, Leydig cells showed an increase in staining intensity which was particularly pronounced in the Leydig cells at weeks 17 and 18. From the 21st week on, a gradual decrease in staining intensity and the number of positive Leydig cells was detectable. In the 33rd week, TrkA-IR was localized to very few single Leydig cells. The similar studies were performed in another literature. The real-time PCR were used to detect expression of NGF in the testis of alpaca from one-month old, 12-months old and 24-months old. One-month old (new-born) alpacas showed significant reduction for NGF mRNA in testis ($P<0.05$) in comparison with both 12-month and 24-month old alpacas, however, 12- and 24-month old alpacas showed no significant differences ($P>0.05$) [8]. In apparent agreement, both p75 and TrkA receptor gene products were detected in immature rat testes, with maximal expression in 10-day-old and 20-day-old rats. However, expression of the p75 and the TrkA receptor were barely detectable in 90-day-old adult rats [9]. Consistently, tow investigations also revealed NGF regulation during Leydig cells development. The research on cattle testis showed that p75 receptor was demonstrated by immunohistochemistry in the 7-month-old fetus and in the early postnatal testis, the peritubular and intertubular fibroblast-like mesenchymal cells showed a strong reaction [10]. Following differentiation of these cells into Leydig cells and myoid peritubular cells [47,48] during postnatal testicular development, the p75 receptor was no longer expressed. This research indicates that NGF plays an important role in adjusting the differentiation and maturation of interstitial cells. Leydig cells were positively stained for NGF, as well as for TrkA and p75LNGFR [24], embryonic testes of TrkA

knockout mice were found to be developmentally delayed and distinguished when compared to their wild-type counterparts [49]. Djakiew et al reported that both the p75LNGFR gene product and the trk receptor gene product were detected in immature rat testes, with maximal expression in 10- and 20-day-old rats. As the mice get older, Expression of the testicular p75LNGFR and the trk receptor progressively declined in older animals, so that they were barely detectable in 90-day-old adult rats [9]. There are two investigations about the postnatal developmental expression of NGF receptors in rat testis, revealing roles of both p75NTR and TrkA during stages of the Leydig cell functional maturation. The highest gene expression of mRNA p75NTR was detected at day 10 or at day 15, whereas highest levels of TrkA transcripts were found in 20-days or 24- to 26-days old animals; Protein examinations revealed the presence of TrkA by immunohistochemical approaches predominantly in Leydig cells [9,50]. In another study from the mouse embryo, at approximately 11.5 days postcoitum (dpc), cells migrate from the mesonephros into the developing testis to contribute to the somatic population of the interstitial compartment (i.e., peritubular myoid cells, Leydig cells, and endothelial cells). Studies from this laboratory have shown that the interstitial population of mesenchymal cells in fetal and newborn mouse testis express the p75 neurotrophin receptor; Cells bearing the p75NTR receptor, purified from 12.5-dpc male mouse mesonephroi by immunomagnetic sorting, were able to differentiate in vitro into myoid cells. suggesting that p75NTR is a marker of the undifferentiated interstitial precursors cell [51].

Physiology levels of testosterone also have shown potential influence on NGF expression from several reports, showing interactions between them. An age-related decrease in serum testosterone levels of 71% between P8 mice 4 and 12 months old, but only a 26% decrease between R1 mice of the same ages in senescence-accelerated mice (SAM-P/8). The level of NGF in testis has a transient increase in SAM-P/8 at around age 4 months old with a subsequent decrease. Consequently, significant differences were apparent in levels of β-NGF between the two types of mouse at ages 2 and 4 months old [52,53,54].

4. NGF and Regulation of Leydig cell function NGF and Its receptors During Leydig cells development

Functionally, as previously described, NGF may act as a differentiation-promoting factor on adult Leydig cells [6]. A lot of reports showed that NGF effect of differentiation-promoting may be involved in Nur77, which is called Orphan nuclear receptor Nur77, also known as NGFI-B in rats and TR3 in humans, is classified as a member of the nuclear receptor superfamily [55]. Nur77 is one of the immediate-early response genes originally identified by virtue of its rapid activation by nerve growth factor (NGF) in PC12 pheochromocytoma cells [56,57]. Nur77 show a very important role in steroidogenesis [58] and they mediate their effects by binding to regulatory elements in promoter regions which referred to expression of Cyp17 gene, HSD3b genes [59] and Star gene [60,61,62]. The studies have shown that LH/ hCG in MA-10 cells induced rapidly NGFI-B/Nur77 gene expression by both CREB and Jun family proteins through the cAMP protein kinase A (PKA) pathway in MA-10 Leydig cells and nerve growth factor (NGF) can induce the phosphorylation of Ser105 of Nur77, resulting in translocation of Nur77 from nucleus to cytoplasm. As aconsequence of the phosphorylation of Ser105 of NGFI-B, LH-mediated steroidogenesis can be strengthened the in MA-10 Leydig cells [58,63]. The mechanism of Leydig cell responsing to NGF may involve in several signal pathway, including Src tyrosine kinase pathway. A cascade of Src and Ras actions, with Src acting first, is a significant feature of the signal transduction pathways for NGF. The Src-Ras cascade may define a functional cassette in the signal transduction pathways used by growth factors and other ligands whose receptors have diverse structures and whose range of actions on various cell types include mitogenesis and differentiation [64]. More importantly, Src tyrosine kinase has an important role in regulating steroid secretion in MA-10 Leydig cells [65]. The regulation may in part be due to Src modulation of phosphodiesterase activity. MA-10 cells expressing a dominant negative Src secreted more cAMP and progesterone in response to LH than control transfected cells. Phosphodiesterase activity was decreased in MA-10 cells. Conversely, MA-10 cells expressing a

temperature sensitive Src lost LH-responsiveness with regard to cAMP and progesterone secretion at the Src active temperature [65].

5. Conclusion

An increasing number of studies suggest that the local NGF activities play a particular role in Leydig cell's steroidogenesis and development. In this complex regulation system, various pathways appear to be involved specifically in the regulation of steroidogenesis processes and in the maintenance of intact spermatogenesis. However, as yet, there are still many uncertain problems. In our previous studies, we found the treatment with the exogenous NGF can increase significantly testosterone plasma levels in aging mouse, but it is well known there is Blood-testis barrier in testis, and many exogenous biological substances can't pass through the barrier, which raise a question that how exogenous NGF perform function on steroidogenesis and spermatogenesis. Therefore, an important goal of future studies will be to more fully understand the involvement of the role of NGF on steroidogenesis of Leydig cell and spermatogenesis and provide further background information for studies on dysfunction of aged Leydig cell, in fact, which may occur, for example, in constellation of andropause symptoms diseases, like PADAM (Partial Androgen Deficiency of the Aging Male).

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