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Null mutations for exon III and exon IV of the p75 neurotrophin receptor gene enhance sympathetic sprouting in response to elevated levels of nerve growth factor in transgenic mice

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Abstract

Under normal conditions, expression of the p75 neurotrophin receptor (p75NTR) by sympathetic neurons can increase the affinity of the signaling receptor, trkA, to target-derived nerve growth factor (NGF) at distal axons. We have previously reported that sprouting of sympathetic axons into NGF-rich target tissues is enhanced when p75NTR expression is perturbed, leading to the postulate that p75NTR may restrain sympathetic sprouting in response to elevated NGF levels. These observations were made using mice having a null mutation of the third p75NTR exon, a line that may express a hypomorphic form of this receptor. Since mice carrying a null mutation of the fourth p75NTR exon may not express a similar splice variant, we sought to determine whether these animals possess the same phenotype of enhanced sympathetic sprouting in response to elevated levels of NGF. Both lines of transgenic mice lacking p75NTR displayed similar degrees of sympathetic axonal sprouting into the cerebellum and trigeminal ganglia, two target tissues having elevated levels of NGF protein. Furthermore, the densities of sympathetic axons in both targets were significantly greater than those observed in age-matched NGF transgenic siblings expressing full-length p75NTR. Our new findings provide a comparative analysis of the phenotype in two independent mutations of the same neurotrophin receptor, revealing that p75NTR plays an important role in restricting sympathetic sprouting in response to higher NGF levels.

Keywords: Neurotrophin; Receptor; Null mutant; Sympathetic sprouting; Mouse

Introduction

Post-ganglionic sympathetic neurons express two transmembrane receptors that bind nerve growth factor (NGF) with differing affinities (Miller et al., 1994; Wyatt and Davies, 1995). High affinity binding of NGF is achieved with the receptor trkA, a 140-kDa protein that mediates intracellular signaling (Kaplan and Stephens, 1994; Kaplan et al., 1991; Klein et al., 1991; Jing et al., 1992; Barbacid, 1995). After NGF binding, trkA receptors homodimerize and subsequently undergo autophosphorylation of tyrosine kinase residues on the cytoplasmic domain of these receptors, which in turn activates multiple downstream effector proteins that mediate neuronal survival and/or neurite elongation (Kaplan, 1998; Kaplan and Miller, 1997, 2000; Kaplan and Stephens, 1994; Greene and Kaplan, 1995; Friedman and Greene, 1999). Neuronal expression of trkA is also necessary for the internalization and retrograde transport of target-derived NGF from axon terminals in peripheral tissues to the cell bodies within the ganglia (Stoeckel et al., 1976; Hendry, 1977; Johnson et al., 1987; Grimes et al., 1996). NGF also binds to the 75 kDa pan-neurotrophin receptor (p75NTR), which has a lower affinity for NGF binding than trkA and lacks kinase activity at its cytoplasmic domain (Bothwell, 1991; Barker, 1998; Friedman and Greene, 1999). Furthermore, unlike trkA, p75NTR expression is not required for neuronal survival or neurite elongation; its role during NGF internalization and retrograde

Abbreviations: BSA, bovine serum albumin; ELISA, enzyme-linked immunosorbant assay; GFAP, glial fibrillary acidic protein; HRP, horseradish peroxidase; IgG, immunoglobulin G; kDa, kiloDalton; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; SCG, superior cervical ganglion; TBS, Tris-buffered saline; TH, tyrosine hydroxylase.

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transport remains unresolved (Weskamp and Reichardt, 1991; Clary et al., 1994; Peng et al., 1995; Lachance et al., 1997). The co-expression of trkA and p75NTR appears to be critical when ligand concentrations are low, such that p75NTR potentiates NGF activation of trkA and collaborates with trkA to form high affinity binding sites, both of which enhance cell survival and neurite outgrowth (Hempstead et al., 1990, 1991; Battleman et al., 1993; Barker and Shooter, 1994; Hantzopoulos et al., 1994; Mahadeo et al., 1994; Gallo et al., 1997; Ryden et al., 1997).

Increasing local levels of NGF stimulates the collateral growth of post-ganglionic sympathetic axons, which may lead to hyperinnervation of target tissues. For instance, transgenic mice overexpressing NGF in the pancreatic islets, skin, heart, lung, and nervous system display a tissue-specific sprouting response by sympathetic fibers (Edwards et al., 1989; Albers et al., 1994; Hassankhani et al., 1995; Kawaja and Crutcher, 1997; Hoyle et al., 1998). As NGF levels in peripheral tissues can be elevated in response to injury or disease (Sebert and Shooter, 1993; Oddiah et al., 1998; Nassenstein et al., 2004), it is unclear whether trkA and p75NTR still co-operate to enhance neuronal responsiveness under these pathological conditions. It has been suggested that p75NTR sequesters excess target-derived NGF at sprouting axon terminals in peripheral tissues (Miller et al., 1994; Coome et al., 1998). By generating mice that overexpress NGF and lack p75NTR function, we found that sympathetic sprouting into those target tissues having elevated levels of NGF protein is enhanced by an absence of p75NTR expression (Hannila and Kawaja, 1999; Walsh et al., 1999a,b). We proposed that p75NTR acts to restrain sympathetic sprouting, such that sequestering excess NGF in target tissues minimizes NGF binding and signaling through trkA. Similar patterns of sympathetic sprouting can be seen in vitro, such that sympathetic neurons isolated from p75NTR null mutant mice display a greater capacity for neurite extension, as compared with sympathetic neurons isolated from age-matched wild type mice (Kohn et al., 1999; Hannila et al., 2004). Enhanced sprouting by p75NTR-deficient sympathetic neurons both in vivo and in vitro may be linked to a greater degree of trkA phosphorylation and subsequent activation of second messenger signals, such as mitogen-activated protein kinases (MAPK) (Hannila et al., 2004).

Numerous studies (Lee et al., 1992, 1994a,b; Bamji et al., 1998; Brennan et al., 1999; Kohn et al., 1999; Majdan et al., 2001), including our own (Coome et al., 1998; Kawaja, 1998; Hannila and Kawaja, 1999; Hannila et al., 2004; Walsh et al., 1999a,b), have used mice carrying two mutated alleles for p75NTR^{exonIII} to assess sympathetic responses to a loss of receptor function; the third exon of p75NTR encodes for the last three of four cysteine-rich repeats in the extracellular domain (Welcher et al., 1991; Yan and Chao, 1991). It later became evident that animals having two mutated alleles for p75NTR^{exonIII} may express a truncated protein product, resulting in the presence of a cleaved intracellular portion of the receptor (von Schack et al., 2001). Although the functional significance of this splice variant remained unknown, there was the possibility that it could alter neuronal responsiveness to NGF, as mutant mice having two mutated alleles for p75NTR^{exonIII} display numerous sympathetic deficits. Dechant and colleagues generated a second mouse model in which the fourth exon for p75NTR is mutated (von Schack et al., 2001); the fourth exon of p75NTR encodes for the transmembrane domain (Chapman and Kuntz, 1995). While these authors reported that mice carrying two mutated alleles for p75NTR^{exonIV} do not express splice variants, a recent study has raised new questions as to whether these mice may likewise express a hypomorphic form of p75NTR (Paul et al., 2004).

Given the fact that two studies have reported the expression of different splice variants of p75NTR in mice carrying null mutations for p75NTR^{exonIII} or p75NTR^{exonIV}, we sought to determine whether these two lines of mice displayed comparable sympathetic features, in particular the ability to sprout new axons in response to elevated levels of NGF. This proof of principal was necessary, as our previous studies revealed an enhanced degree of sympathetic axonal arborization in mice having two mutated alleles for p75NTR^{exonIII}, as compared with wild type mice (Hannila and Kawaja, 1999; Walsh et al., 1999a, b). Our new data reveal that sympathetic neurons of mice carrying null mutants for either p75NTR^{exonIII} or p75NTR^{exonIV} exhibit similar morphological features and phenotypes, including comparable levels of trkA expression. Importantly, in the absence of full-length p75NTR expression, both independent lines of mice display similar patterns of NGF-mediated sprouting response by sympathetic axons in vivo, which are significantly greater than those detected in age-matched wild type siblings. These data further corroborate the idea that, in the absence of p75NTR function, sympathetic axons undergo an enhanced degree of terminal arborization in response to elevated levels of NGF. Thus, p75NTR acts to regulate the collateral growth of NGF-sensitive sympathetic axons, in particular when local levels of NGF may be elevated due to disease or damage of target tissues.

Materials and methods

Animals

New lines of hybrid mice were generated by breeding female mice carrying one mutated allele for p75NTR^{exonIII} (Lee et al., 1992) or p75NTR^{exonIV} (von Schack et al., 2001) with male transgenic mice that overexpress NGF under the control of the promoter for glial fibrillary acidic protein (GFAP) (Kawaja and Crutcher, 1997). Progeny from these and subsequent crosses were genotyped by polymerase chain reaction. Briefly, tail DNA isolated from 4-week old mice was digested with *Eco*RI and amplified with the following primers: p75NTR^{exonIII} (5′/WT: 5′-GTG TTA CGT TCT CTG ACG TTG TG; 3′/WT: 5′-TCT CAT TCG GCG TCA GCC CAG GG; and 3′/Neo: 5′-GAT TCG CAG CGC ATC GCC TT); p75NTR^{exonIV} (5′/WT: 5′-GAT GGA TCA CAA GGT CTA CGC; 3′/WT: 5′-TGT TGG AGG ATG AAT TTA GGG; 3′/Neo: 5′-AAG GGG CCA CCA AAG AAC GG); and transgenic NGF (5′-CTAGAA TTC TGC AGG CAA GTC AGC C; 5′-CCT GAA TTC TAG TGA ACA TGC TGT GCC). Those animals expressing the NGF transgene and