Nerve Growth Factor and Pain Mechanisms

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Annu. Rev. Neurosci. 2017. 40:307–25

First published as a Review in Advance on April 24, 2017

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

https://doi.org/10.1146/annurev-neuro-072116-031121

Keywords

nerve growth factor, chronic pain, trkA, p75

Abstract

Nerve growth factor (NGF) antagonism is on the verge of becoming a powerful analgesic treatment for numerous conditions, including osteoarthritis and lower back pain. This review summarizes the historical research, both fundamental and clinical, that led to our current understanding of NGF biology. We also discuss the surprising number of questions that remain about NGF expression patterns and NGF's various functions and interaction partners in relation to persistent pain and the potential side effects of anti-NGF therapy.

Contents

1. INTRODUCTION

Nerve growth factor (NGF) was first described in studies dating back more than half a century, when, as its name suggests, it was recognized as a substance promoting neuronal growth in developing chick embryos (Bueker 1948, Levi-Montalcini & Hamburger 1951). The protein was subsequently purified (Cohen 1960), and only much later was it recognized that this was one of a family of structurally related proteins that also comprise, in mammals, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4-5). All are secreted proteins that promote survival and growth of different elements of the peripheral nervous system.

In the past few decades, it has become clear that these factors have two different roles, in development and in maturity. The developmental role is a critical one for the survival of different cells of the peripheral nervous system, but the adult role is the main focus of this review. And that is that one of these proteins, the prototypical neurotrophin, NGF, is an important pain mediator for which we now have very extensive evidence. We are on the threshold of seeing the emergence of what appears to be a powerful new class of analgesic drug with multiple applications based on NGF antagonism. It is this story that we follow here.

2. NEUROTROPHINS AND THEIR RECEPTORS

Neurotrophins can bind two kinds of receptors: tyrosine receptor kinases known as trks and a member of the tumor necrosis factor receptor superfamily known as $p75^{NTR}$. There are three different trk receptors (trkA, trkB, and trkC), each with its own preference for particular neurotrophins. trkA is highly selective for NGF and, to a lesser extent, NT-3, whereas trkC binds NT-3, trkB, BDNF, and NT-4/5. In contrast, $p75^{NTR}$ is promiscuous, binding all neurotrophins, including NGF, at low affinity.

Our understanding of NGF receptor signaling initially seemed straightforward (Chao & Hempstead 1995, Greene & Kaplan 1995) (see **Figure 1** for an example): On the one hand, NGF was seen as having high affinity and biologically very important binding to trkA, which then homodimerizes, autophosphorylates, and activates downstream second-messenger cascades, such as phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)–protein kinase B (Akt), extracellular signal–related kinases, or phospholipase Cγ. This path was thought to promote neuronal

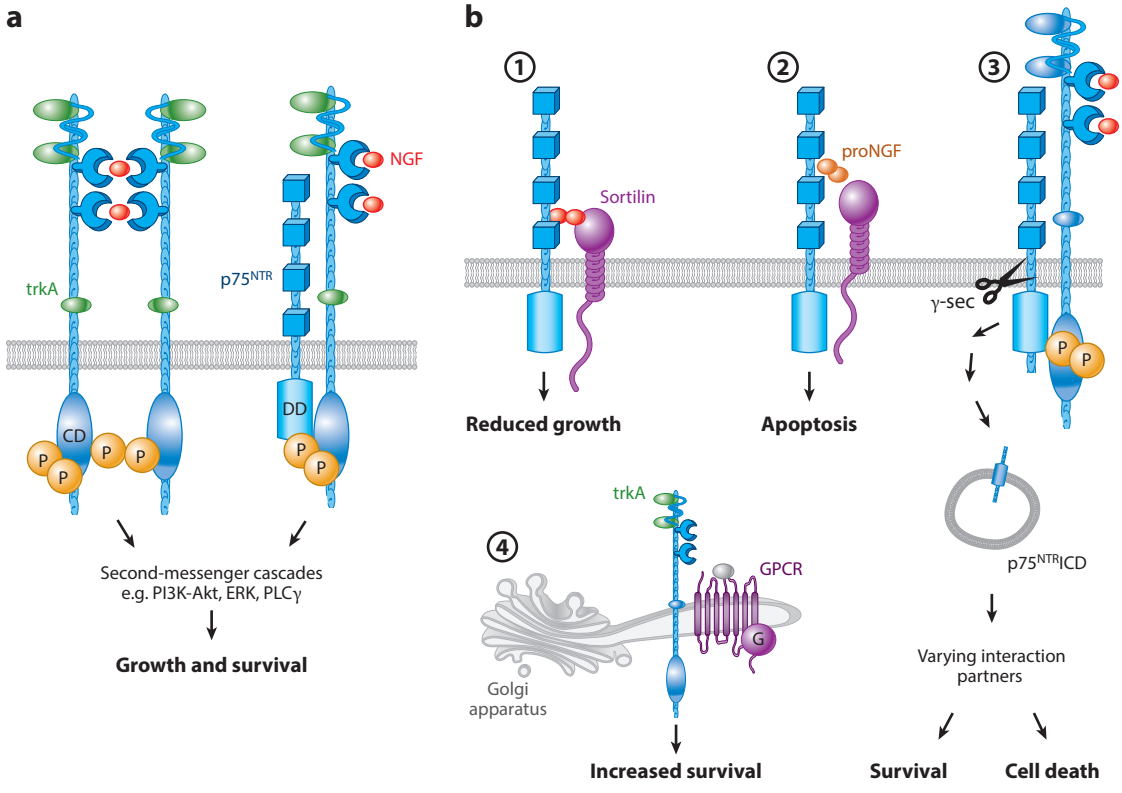

Figure 1

Neurotrophins and their receptors. (*a*) Traditionally, NGF was known to bind two receptor constellations: trkA homodimers (*left*) or trkA-p75^{NTR} heterodimers (*right*). Either leads to phosphorylation of the cytoplasmic end of the trkA receptor and the triggering of various different second-messenger cascades that affect growth and survival processes within the cell. (*b*) More recently, our understanding of NGF signaling has become more complex: NGF can also bind Sortilin/p75^{NTR} to reduce growth (①); a proform of NGF (proNGF) is released extracellularly and can bind NGF receptor complexes (e.g., p75^{NTR}-Sortilin) that induce apoptosis (②); the ICD of p75^{NTR} is cleaved by γ-sec, is packaged into endosomes, and can then bind varying interaction partners inside the cell to effect opposing functions, such as survival and cell death (③); and finally, trkA can be transactivated by GPCRs, increasing cell survival—a process that has been suggested to take place intracellularly within the Golgi apparatus (④). Abbreviations: γ-sec, γ-secretase; CD, catalytic domain; DD, death domain; GPCR, G protein–coupled receptor; ICD, intracellular cytoplasmic domain; NGF, nerve growth factor; PI3K-Akt, phosphatidylinositol-4,5-bisphosphate 3-kinase–protein kinase B; PLCγ, phospholipase Cγ.

survival. On the other hand, NGF could also bind p75^{NTR}, albeit at low affinity. Lacking a catalytic domain, p75^{NTR} has to dimerize with trkA, and there is good evidence that the two receptors act synergistically, for instance to enhance growth (Bibel et al. 1999, Hempstead et al. 1991).

Over time, however, our understanding of NGF signaling has increased significantly, adding several layers of complexity (see, e.g., Kelleher et al. 2016, Lewin & Nykjaer 2014, Meeker & Williams 2014 for reviews): (*a*) p75^{NTR} can form heterodimers with a variety of other auxiliary receptors, most prominently Sortilin and neurite outgrowth inhibitor reticulon 4 (NOGO); (*b*) equally, trkA can be transactivated by other G protein–coupled receptors without the involvement of NGF; (*c*) intracellularly, the receptors can apparently be processed in many different ways—potentially leading to distinct functional outcomes; and finally, (*d*) a proform of NGF (proNGF), previously thought to be a simple byproduct of NGF production, appears to be released extracellularly and is capable of preferential binding to p75^{NTR}.

What does all this new information mean for our understanding of NGF signaling, especially in the context of nociceptive signaling in health or disease? So far, the evidence is still patchy and often focused on growth and development rather than mature systems, where we know that NGF function is likely to be different.

There seems to be little doubt that activation of Sortilin/p75^{NTR} heterodimers can negatively affect sensory neuron growth (Hempstead 2006). For instance, elegant work has been conducted on global, constitutive Sortilin/p75^{NTR} double knockouts showing that they display a decrease in both mechanical and thermal sensitivity as compared to single gene knockout mice (Vaegter et al. 2011). This loss of sensation was due to a reduction in peripheral neuron numbers, including the degeneration and loss of unmyelinated C fibers. However, it is still unknown what effect Sortilin dysfunction might have on nociception in the context of normal sensory neuron development. Sortilin knockout mice display reduced anterograde trkA transport (Vaegter et al. 2011), raising the possibility that modulation of the receptor could dampen NGF-mediated sensitization. But we lack concrete evidence as yet. Indeed, even basic questions, such as the proportion of Sortilin molecules located in extra- versus intracellular membranes, remain unanswered (Lewin & Nykjaer 2014).

Similarly, the cross talk between trkA and other signaling pathways (transactivation) is poorly understood in the context of nociception. It appears that G protein–coupled receptor ligands, such as adenosine and pituitary adenylate cyclase-activating polypeptide, can activate trk receptors and increase neuronal survival (Rajagopal et al. 2004). This process mostly appears to be limited to intracellularly localized trkA, at least in the cell line and embryonic cortical neurons in which it has been studied. Indirect evidence suggests that transactivation can also occur in dorsal root ganglion (DRG) neurons (Shi et al. 2009), but whether this plays a role in chronic pain is unknown. So far, interest in the biological purpose of trkA transactivation has been mainly concerned with neuronal survival as opposed to postdevelopmental sensitization events (Chao 2003).

At the intracellular level, effort has gone into testing whether one might be able to target different signaling pathways, affecting NGF-induced hypersensitivity without interfering with its beneficial, neurotrophic function. This has received particular attention since it became known that anti-NGF antibodies may have unwanted side effects, as reviewed below. p75^{NTR}, for instance, is cleaved upon activation, and the resulting intracellular domain is transported via endosomes to many different locations in the cell. Its actions can therefore be as wide-ranging as its interaction partners, from growth promotion via trkA to apoptosis via Sortilin (Skeldal et al. 2011). Similarly, trkA receptors are transported around the cell and, as discussed below, can even travel retrogradely along axons to affect nuclear transcription (Ginty & Segal 2002). Despite our increasing understanding of these processes, the ubiquitous nature of many intracellular signaling cascades likely means that selectivity for drug development will remain elusive.

Finally, researchers are increasingly interested in the role of proNGF, rather than the mature form. Since the first report of its extracellular release (Lee et al. 2001), evidence has accumulated to suggest that proNGF binds p75^{NTR} selectively, although there are some contradictory data as to whether the subsequent intracellular signals can lead to trkA phosphorylation (Boutilier et al. 2008, Howard et al. 2013, Watanabe et al. 2008). ProNGF has been reported variably to induce apoptosis via Sortilin/p75^{NTR} binding (Nykjaer et al. 2004) or promote outgrowth of dorsal root and sympathetic ganglion neurons—an effect that is abolished in p75^{NTR} knockout mice (Howard et al. 2013). Finally, and maybe most relevant to the study of chronic pain, breaking down extracellular proNGF using the protease plasmin apparently decreases hyperalgesia in animals, whereas intraplantar injection of proNGF causes mechanical hypersensitivity, implying a pronociceptive role for the proform, too. There has also been a report of a role for proNGF produced in activated microglia (Yune et al. 2007)—a less convincing result, however, given that several recent RNA sequencing (RNA-seq) studies clearly show microglia to be entirely devoid of mRNA for

NGF (Gosselin et al. 2014, Lavin et al. 2014), even after nerve injury (Denk et al. 2016). When interpreting results pertaining to proNGF versus NGF signaling, it is important to bear in mind that distinguishing these two forms is not an easy task at present. Available immunoassay tools are unspecific (Malerba et al. 2016), including the molecules being tested in the clinic, which we discuss further below. But first, we give a summary of the endogenous role of NGF in nociception, touching briefly on development, expression patterns, and finally biological function in the adult peripheral nervous system.

3. DEVELOPMENTAL ROLE OF NEUROTROPHINS

During development, an excess of sensory neurons are generated and grow axons toward peripheral targets. The density of peripheral innervation is supported by the limited availability of neurotrophic factors, a concept originally known as the neurotrophic hypothesis (Davies 1988, 1996). The details of this process have been reviewed extensively elsewhere (e.g., Marmigere & Ernfors 2007), but there are some key points to remember for the purpose of this review. Nociceptive sensory neurons essentially all require NGF for survival during development, but this dependence is lost 1–2 weeks postnatally in rat or mouse. During this time, about 50% of sensory neurons lose their expression of trkA and switch to expressing receptors for glial cell–derived neurotrophic factor (**Figure 2**). Notably, sympathetic postganglionic neurons also express trkA from early in development (Aloe et al. 1975) and throughout adulthood, and there has been some debate about whether these cells, in contrast to DRG neurons, continue to need NGF for survival in maturity. The current consensus suggests that this is not the case (see below).

The implications for targeting NGF in humans is clear (**Table 1**): If this is done early in development, one would expect the peripheral pain-signaling and sympathetic systems to fail to develop. This indeed is the case in rare genetic mutations associated with loss-of-function of trkA

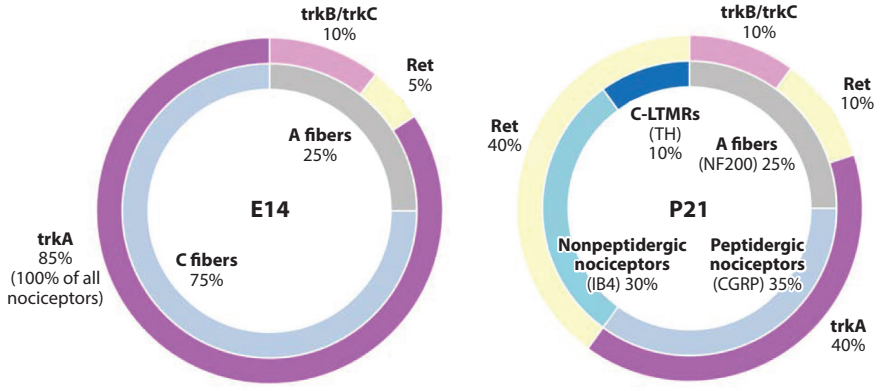

Figure 2

DRG neuron subtype and receptor distribution in development and adulthood. During development (E14), the vast majority of DRG sensory neurons and all nociceptors express trkA, whereas only a subgroup express the receptors for BDNF (trkB), NT-3 (trkC), and glial cell–derived neurotrophic factor (Ret). Postnatally, this ratio shifts in favor of Ret expression, with approximately half of all nociceptors losing their trkA expression and hence their dependence on NGF. Abbreviations: BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide, a marker of peptidergic nociceptors; DRG, dorsal root ganglion; E14, embryonic day 14; IB4, isolectin B4, a marker of nonpeptidergic sensory neurons; NF200, neurofilament 200, a marker for myelinated neurons; NT-3, neurotrophin-3; P21, postnatal day 21; TH, tyrosine hydroxylase, a marker of low-threshold C fiber mechanoreceptors (C-LMTRs).

Table 1 Summary of effects of trkA/NGF modulation on sensory neurons[a]

Species	Intervention	Developmental age	Effect on nociceptors	Effect on sympathetic neurons	Functional effects
Rat/mouse	trkA/NGF knockout	<P10	Complete loss[b]	Complete loss[b]	Analgesia and anhydrosis
Rat/mouse	trkA/NGF knockout	>P10	No change[c]	No change[c]	No change in basal sensitivity[c]
Human	trkA/NGF loss-of-function	Embryonic	Complete loss[b]	Complete loss[b]	Analgesia and anhydrosis
Human	Anti-NGF treatment	Adult (up to 24 weeks after treatment)	No change in epidermal innervation	No change[b]	Basal sensitivity: no change[c] Chronic pain: significant improvement

Abbreviations: NGF, nerve growth factor; P10, postnatal day 10.

[a]The data illustrate the divergent roles of NGF and its receptor during development and adulthood.

[b]More or less.

[c]The literature has not yet come to a firm conclusion.

[d]This relates to functional measures. Morphometry has not been assessed in humans.

or NGF (Einarsdottir et al. 2004, Indo et al. 1996, Klionsky et al. 2012, Minde et al. 2009). Patients have almost no C fibers and reduced Aδ fiber populations in their peripheral nerves, do not feel pain, and cannot sweat. Similarly then, giving drugs that target NGF or NGF signaling may have the same consequences in early development (probably up to a few years of age in humans) while leaving adults unaffected. So far, one study in pregnant monkeys supports this hypothesis (Bowman et al. 2015) (see more below).

There is also an interesting, very rare clinical example of people with a mutation in the *NGF* gene resulting in a form of proNGF that is resistant to cleavage (Einarsdottir et al. 2004, Larsson et al. 2009). These individuals are likely therefore to have excess levels of proNGF and reduced levels of mature NGF throughout development. Patients display some of the developmental consequences of loss of NGF, with reduced survival of peripheral nociceptors. To what extent their phenotype reflects the absence of mature NGF versus the overexpression of proNGF is, at present, unclear.

4. ADULT EXPRESSION OF RECEPTORS AND LIGANDS

The above section makes some issues clear. Firstly, about one-half of adult nociceptors continue to express trkA and $p75^{NTR}$. Some of these also express other neurotrophin receptors. Of note, many visceral afferents appear to coexpress trkA and trkB (McMahon et al. 1994), and some other nociceptors may have low levels of trkC (Thakur et al. 2014, Verge et al. 1992). There are also truncated versions of trkB that lack signaling domains. These are expressed in many DRG neurons, but their role is unknown. These patterns could change with pathology, but the most noticeable change reported to date is a downregulation of trkA (and trkC) and $p75^{NTR}$ in sensory neurons after nerve injury (McMahon et al. 1994, Verge et al. 1992).

What about neurotrophin receptors outside of sensory neurons? These of course could affect sensory events indirectly. Here, the advent of large-scale RNA-seq studies provides a lot of data, much of it at variance with earlier immunohistochemical reports (obtained frequently with antibodies that were not knockout validated). Contrary to some of those, trkA is absent from myeloid cell populations and remains so even upon their stimulation with a variety of agents (Denk et al. 2016, Gosselin et al. 2014, Lavin et al. 2014, Ostuni et al. 2013). Similarly, there is no trkA on bone marrow–derived mast cells (Calero-Nieto et al. 2014, Chen et al. 2015, Hutchins et al. 2015)

or keratinocytes (Cavazza et al. 2016, Farshchian et al. 2015, Rinaldi et al. 2016). There is trkA in some nonneuronal cells in the DRG (Thakur et al. 2014), and this may increase in some neuropathic pain models (E.J. Bradbury, unpublished observation). In terms of neuronal expression, it seems that trkA is restricted specifically to peripheral nociceptors and is mostly absent from the central nervous system (CNS) (Mardinly et al. 2016, Mo et al. 2015, Thakurela et al. 2016), with the exception of basal forebrain cholinergic neurons (Sanchez-Ortiz et al. 2012).

$p75^{NTR}$ is expressed more widely. It is expressed in DRG satellite cells (Thakur et al. 2014) and Schwann cells (Bentley & Lee 2000, Ramer et al. 1999, Tomita et al. 2007) and can potentially be found at low levels in differentiated keratinocytes (Cavazza et al. 2016, Farshchian et al. 2015). Macrophages, even after stimulation with a host of mediators (IL-1β, tumor necrosis factor-α, IFN-γ, IL-4, lipopolysaccharide, and IL-10), show negligible amounts of message (Hutchins et al. 2015, Ostuni et al. 2013). In mast cells, the data are more contradictory: The data sets range from zero expression to low-level expression to unexpectedly high expression (Calero-Nieto et al. 2014, Chen et al. 2015, Hutchins et al. 2015). Partly, the confusion might arise as a consequence of the cell extraction method. In the nervous system, there are, as said above, high levels of $p75^{NTR}$ in DRG and sympathetic ganglia but much lower levels in the CNS, at least under normal circumstances.

One little-appreciated mystery is where NGF is actually being produced in the naive adult organism. NGF was reported to be made by mast cells (Leon et al. 1994), macrophages (Barouch et al. 2001), and keratinocytes (Lewin & Nykjaer 2014). However, RNA-seq studies again suggest that NGF transcripts are virtually absent from both mouse bone marrow–derived (Calero-Nieto et al. 2014, Chen et al. 2015, Hutchins et al. 2015) as well as human peripheral blood–derived mast cells (Kanemaru et al. 2015), at least in their naive, quiescent state. Moreover, human keratinocytes also lack NGF expression (Cavazza et al. 2016, Farshchian et al. 2015, Rinaldi et al. 2016). Indeed, the list of pure cell types from which NGF message is absent is very long and includes every single human blood cell type analyzed by the BLUEPRINT Consortium; any resident myeloid cell type, including microglia (Denk et al. 2016, Gosselin et al. 2014, Lavin et al. 2014, Ostuni et al. 2013); mouse peripheral sensory neurons (Reynders et al. 2015, Thakur et al. 2014, Usoskin et al. 2015); human trigeminal and dorsal root ganglia (Flegel et al. 2015); and cortical myelin (Thakurela et al. 2016).

Although in normal conditions in the adult, NGF appears to be little expressed, there is evidence that NGF protein levels are elevated in multiple pathological states, most commonly those associated with inflammation (see below). Which cells start producing NGF in this context? The early literature again suggests multiple cell types including, but not limited to, keratinocytes (Fujimoto et al. 2008, Tron et al. 1990), mast cells (Lewin et al. 1994, Woolf et al. 1996), and macrophages (Mantyh et al. 2011). More recent RNA-seq data collected by Ostuni and colleagues (Hu et al. 2016) also suggest that bone marrow–derived macrophages express NGF specifically after stimulation with IFN-γ and potentially also after administration of Malp2 and IL-1β. By contrast, and more in line with received wisdom, nerve injury does not cause upregulation of NGF in peripheral sensory neurons.

In summary, our understanding of the precise, cell type–specific expression patterns of NGF and its receptors may have to be redefined in some instances. Given that NGF has become such a promising drug target, our knowledge appears to be surprisingly patchy. This is in contrast to a much more well-defined functional role of NGF in nociception, which we discuss in the following section.

5. BIOLOGICAL EFFECTS OF NGF ON NOCICEPTIVE PROCESSING

NGF injected into healthy human skin can produce localized pain and hyperalgesia that develops within minutes (Petty et al. 1994), suggesting an activating or sensitizing effect on nociceptors at

the injection site. Systemic injections of even low doses of NGF (above 1 μg/kg) could also result in what the authors called myalgia—a widespread, deep musculoskeletal pain affecting proximal body regions and reminiscent of the sensory disturbances observed frequently with mild infections. The most parsimonious explanation is that NGF can also sensitize deep somatic afferents. All these findings in humans are consistent with the notion that NGF acts as a pain mediator in mature mammals. The mechanisms of NGF-induced pain and hyperalgesia have been investigated extensively in laboratory studies. Indeed, anti-NGF therapy is one of the few instances in drug development where preclinical work led to a promising new target for patients. We would argue that although the rat and mouse models that are used in the field are far from perfect—being mostly limited to measures of evoked pain—they can clearly yield important insights into the biology of prospective treatment approaches, as they did in the case of NGF. The success of the preclinical approach in this instance was likely due to the sheer wealth and robustness of the underlying data and the detailed biological insights they yielded. The conclusion of those many studies is that NGF may interact with nociceptors in three prominent ways, as described below.

5.1. NGF-Induced Sensitization of Peripheral Nociceptive Terminals

Adult primary sensory nociceptive neurons from rodents can be maintained in culture in a defined medium. Application of NGF to these cultures results in activation of trkA and the rapid sensitization of those neurons to a variety of stimuli, including noxious heat (Zhang et al. 2005), mechanical stimuli (Di Castro et al. 2006), or chemical stimuli such as capsaicin (Winter et al. 1988) (c.f. Basbaum et al. 2009 for a review). The mechanism of this effect is still a little uncertain, with a claimed essential role for PI3K and mitogen-activated protein kinase (Bonnington & McNaughton 2003, Cao et al. 2013, Stein et al. 2006), protein kinase C γ and phosphatidylinositol 4,5-bisphosphate (PIP2) (Chuang et al. 2001), or protein kinase A (Shu & Mendell 2001). These cascades increase both phosphorylation and trafficking of transient receptor potential cation channel subfamily V member 1 (trpV1), which accounts for some of the sensitizing effects. However, there is also evidence for regulation of other receptors and ion channels (Mizumura & Murase 2015).

Modulation of NGF, either via administration in vivo or genetic modification in a skin nerve preparation, also results in sensitization in an apparently similar manner (Rueff et al. 1996, Stucky et al. 1999). A role for trpV1 in this context has once again been demonstrated elegantly in a recent study in the naked mole rat, in which reduced trkA function was shown to decrease trpV1 sensitization and subsequent NGF-induced hyperalgesia (Omerbasic et al. 2016). There have also been several suggestions from in vivo studies that some of the sensitizing effects of NGF may be mediated indirectly, via immune or other cells in the tissue that are NGF sensitive. However, as we review above, recent expression data do not support the idea that many cells express at least trkA, and so this idea needs critical reevaluation.

5.2. NGF-Induced Altered Nociceptor Transcription

It is well established that NGF binding to trkA is followed by endocytosis of the ligand-receptor complex and retrograde transport of the same in signaling endosomes (Ginty & Segal 2002). The nuclear effects of increased NGF retrograde transport include transcriptional regulation of many important nociceptor genes. Somewhat surprisingly, we do not have a genome-wide analysis of NGF-sensitive genes, but candidate gene analysis has revealed a clear modulating influence of NGF on the expression levels of several nociceptive receptors (such as trpV1, P2X3, and ASIC3), transmitters (such as substance P, calcitonin gene-related peptide, and BDNF), and ion

channels (including several Na_vs and Ca_vs). Schmelz and colleagues have used pig DRGs (Jonas et al. 2015, Petersson et al. 2014) and human skin (Rukwied et al. 2010), as well as computational algorithms (Petersson et al. 2014, Tigerholm et al. 2014), to model human sensory neuron function. Their data suggest that low-dose injections of NGF subcutaneously can modulate some of the electrophysiological properties of nociceptors with a latency of several weeks, strongly suggesting a transcriptional mechanism. These electrophysiological properties reflect the excitability of nociceptors and are therefore likely to be functionally important.

5.3. NGF-Induced Sprouting of Nociceptors

In addition to regulation of nociceptive genes, as discussed above, NGF can clearly also induce or promote a program of gene expression related to axonal growth (Zhou et al. 2004). This, too, may have consequences for nociceptive processing. This anatomical growth is very apparent in several clinical states. One is in osteoarthritis, in which healthy cartilage is usually aneural (Lane et al. 1977). However, in diseased tissue, sensory and sympathetic nerve fibers are often found, and NGF-induced growth is the most parsimonious explanation (because NGF levels are known to be increased in these states). The other example is in bone cancer pain. The group of Mantyh (Mantyh 2014) has developed models of bone cancer in experimental animals with high face validity. One of their findings is a rather remarkable remodeling of afferent neuron anatomy. Bone-harboring carcinoma cells become dramatically hyperinnervated, and this is NGF dependent. This mechanism (nociceptor hyperinnervation) is likely to be present in other chronic pain conditions but has not been investigated extensively. The sensitivity of any nociceptor sprouts is not known, nor is the total contribution of this mechanism to chronic pain in general.

6. NGF AS A THERAPEUTIC TARGET FOR CLINICAL PAIN STATES

As outlined in the above section, NGF has a profound and long-lasting sensitizing effect on the nociceptive system in both rodent and humans, but is this linked to human pain states? The levels of NGF mRNA and protein are increased in several human pain disorders, especially in the context of inflammation; this has been shown in both patient-derived samples and experimental pain models. Examples include bladder pain syndrome/interstitial cystitis (Chen et al. 2016), inflammatory bowel disease (di Mola et al. 2000), chronic pancreatitis (Friess et al. 1999), osteoarthritis (Iannone et al. 2002), rheumatoid arthritis and spondyloarthritis (Aloe et al. 1992, Barthel et al. 2009), and the human UV burn model (Dawes et al. 2011). In some cases, this increased NGF expression was observed in epithelial cells within in the tissue; however, in other situations, this increased expression is claimed to be from resident and infiltrating immune cells such as mast cells and macrophages (but see caveats on expression, above).

Chronic pain represents a huge health and indeed societal problem, affecting 1 in 5 people. It is associated with multiple comorbidities such as depression and has a significant negative impact on quality of life and employment (Breivik et al. 2006). Current treatments for chronic pain are limited by inadequate efficacy, poor tolerability, and abuse potential [especially in the use of opiates in nonmalignant pain (Mitra 2013)]. Novel analgesics are therefore urgently needed. To this end, there has been growing interest in targeting NGF and its downstream signaling pathways to provide analgesia.

Data from preclinical pain models proved encouraging in this regard, suggesting that antagonism of NGF can ameliorate pain-related behavior. Thermal and mechanical pain–related hypersensitivity evoked by paw inflammation (using complete Freund's adjuvant or carrageenan) could be blocked using either antibodies to NGF (Lewin et al. 1994, Woolf et al. 1994) or a

trkA-IgG molecule to sequester NGF (McMahon et al. 1995). Anti-NGF therapy produced analgesia but had no effect on tissue swelling. As such, this treatment does not impact the inflammatory response per se but rather impacts the downstream nociceptor sensitization. Anti-NGF has now also been shown to reduce pain-related hypersensitivity in diverse preclinical pain models of human chronic pain disorders including bone cancer pain (Sevcik et al. 2005), plantar incision as a postsurgical pain model (Zahn et al. 2004), and a bone fracture model of complex regional pain syndrome (Sabsovich et al. 2008).

Several mechanisms have been proposed by which anti-NGF may decrease pain-related hypersensitivity. One is a reduction in the spontaneous activity and the leftward shift in the stimulus-response function of nociceptive afferents evoked by inflammation (Koltzenburg et al. 1999). Anti-NGF can also normalize the expression of neuromodulators by primary afferents, which is increased by inflammation (McMahon et al. 1995, Woolf 1996). Finally, in certain contexts, anti-NGF may have a disease-modifying effect relating to the excessive axon outgrowth described above. In models of bone cancer pain and inflammatory arthropathy, localized sprouting of small fibers (both nociceptive afferents and sympathetic fibers) is prevented by anti-NGF treatment (Ghilardi et al. 2012, Sevcik et al. 2005) (see **Figure 3**).

Given that lack of NGF has been proposed as a causal factor in some peripheral neuropathies [notably diabetic neuropathy (Apfel et al. 1994)], scientists and regulators are cautious about reducing NGF levels to treat peripheral neuropathic pain for fear of exacerbating a preexisting neuropathy. However, evidence suggests that in some patients suffering from peripheral neuropathy, NGF levels may be locally increased (Anand 2004), providing a rationale for normalizing such levels. Indeed, there are preclinical data for the efficacy of anti-NGF in rodent neuropathic pain models (Dos Reis et al. 2016, Wild et al. 2007).

These encouraging preclinical and clinical data provided a strong rationale to drug development programs aimed at targeting NGF and subsequent clinical trials. These trials have used a variety of different approaches. Given the increasing expertise in biological therapies, several companies have developed humanized monoclonal antibodies to bind to NGF with high specificity and affinity, preventing interaction with its receptor. These include tanezumab (Pfizer and Eli Lilly), fasinumab (Regeneron and Teva), and fulranumab (Janssen and Amgen). The homology between different trk receptors has meant that developing a specific small-molecule antagonist of trkA (which does not bind to trkB and trkC) has been more of a challenge, but it would have the significant advantage of an agent that could be used orally. Concern regarding a pan-trk inhibitor would relate to the potential marked CNS side effects of trkB inhibition. One potential means of ameliorating this would be to generate peripherally restricted compounds. Several pan-trk inhibitors have been developed that do not inhibit other kinases (for instance, see Stachel et al. 2014), and such agents are now entering first-in-human studies (see **https://clinicaltrials.gov/ct2/show/NCT02454387**).

A series of positive trials in the treatment of osteoarthritis provided an impetus to use anti-NGF as an analgesic. Osteoarthritis is the most common joint disorder worldwide (affecting 10% of men and 18% of women over 60), is a common cause of chronic pain, and will become more prevalent given the aging population (Glyn-Jones et al. 2015). Humanized anti-NGF monoclonals have now been developed and used in multiple randomized placebo-controlled trials (RCTs) for the treatment of painful osteoarthritis. The first positive report was of tanezumab, which showed analgesic efficacy in comparison to placebo in a Phase II RCT in the treatment of knee osteoarthritis (Lane et al. 2010) (see **Figure 4**).

Schnitzer & Marks (2015) recently conducted a systematic review of 13 high-quality Phase II and III RCTs that used these monoclonals in knee or hip osteoarthritis pain. This noted that all three anti-NGF monoclonals showed efficacy in reducing pain and improving function over placebo and that tanezumab had greater efficacy when compared to nonsteroidal anti-inflammatory

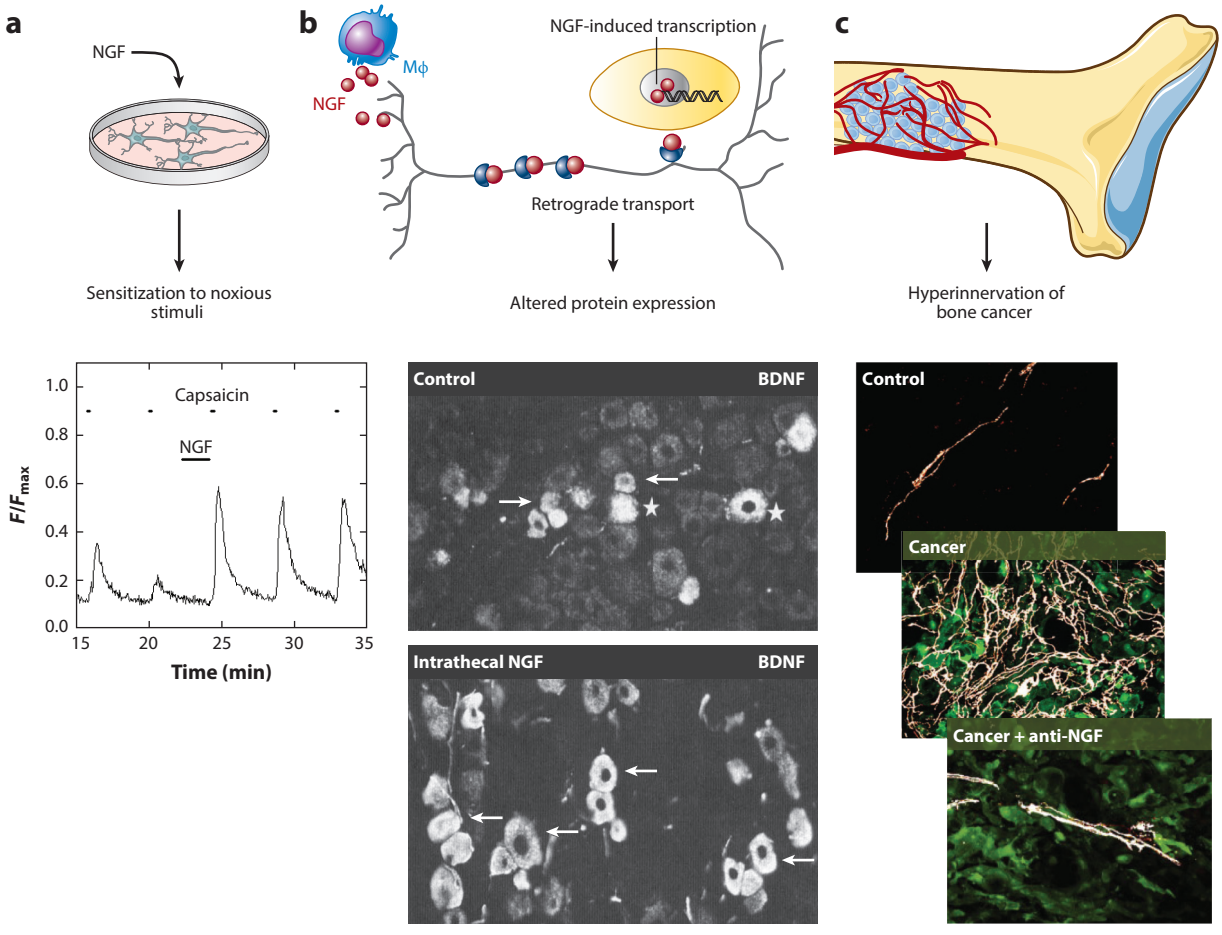

Figure 3

Biological effects of NGF on nociceptive processing. NGF is known to have at least three consequences on sensory neuron function. (*a*) NGF causes peripheral sensitization both in vitro and in vivo, as illustrated by the increased response of DRG neurons to capsaicin in its presence. Panel courtesy of Prof. Peter McNaughton. (*b*) NGF leads to transcriptional regulation after retrograde axonal transport, as illustrated by immunostaining showing upregulation of BDNF after intrathecal NGF treatment (Michael et al. 1997). Pictures reprinted with permission from the *Journal of Neuroscience*. (*c*) Finally, NGF can cause sprouting of peripheral afferents into diseased joints and cancerous tissue. Images are courtesy of Prof. Patrick Mantyh and show CGRP staining innervating a mouse femoral bone in a control condition, a cancerous condition, and a condition in which anti-NGF antibody has been delivered to the animal. Abbreviations: BDNF, brain-derived neurotrophic factor; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; Mϕ, macrophage; NGF, nerve growth factor.

drugs (NSAIDs) and opiate treatment. There is less experience in the use of anti-NGF therapy in the common problem of low back pain; however, a trial using tanezumab reported improved efficacy of tanezumab versus placebo and NSAIDs in reducing pain scores and disability (Kivitz et al. 2013). A small RCT of tanezumab has also been conducted in painful diabetic neuropathy and postherpetic neuralgia (PHN). Tanezumab had significant efficacy in treating neuropathic pain due to diabetic neuropathy but not PHN. Reassuringly, there was no evidence of neuropathy progression in the tanezumab-treated arm (Bramson et al. 2015).

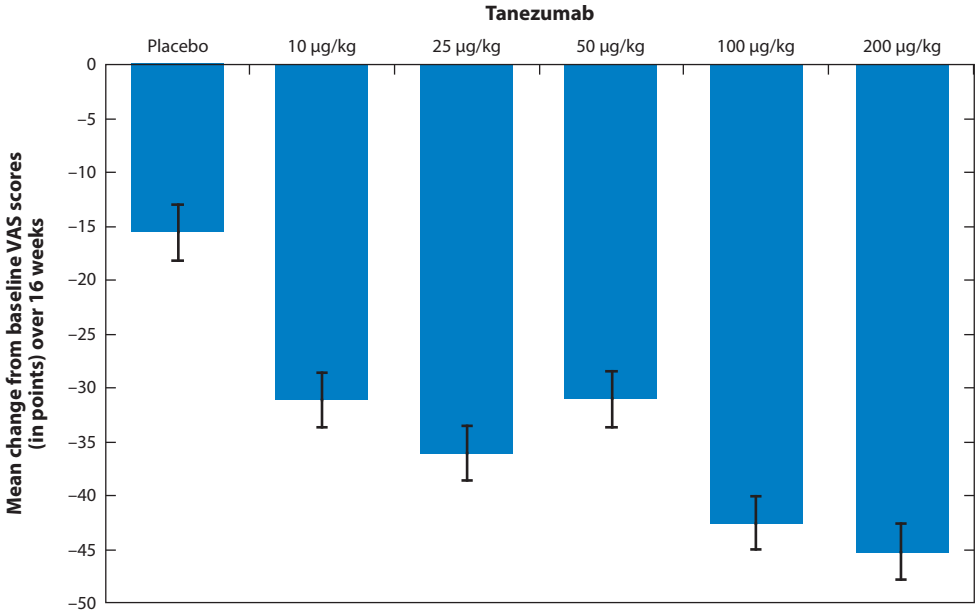

Figure 4

Anti-NGF treatment with tanezumab has a therapeutic effect in humans. Data extracted from Lane et al. (2010) show a reduction in knee pain in a cohort of 450 osteoarthritis patients enrolled in a Phase II randomized controlled trial for the humanized anti-NGF monoclonal antibody tanezumab. The patients entered the study with an average pain score of approximately 65 [on a 100-point visual analog scale (VAS)], and so the higher doses of anti-NGF exerted a profound analgesic effect.

The convincing findings that anti-NGF has potent analgesic effects are encouraging and provide one of the few examples whereby a molecular target identified and validated in preclinical research has subsequently shown efficacy in clinical trials. In 2010, the US Food and Drug Administration (FDA) called a halt initially to tanezumab and then to all clinical trials using anti-NGF monoclonals because a small subset of patients developed rapidly progressive joint degeneration (FDA 2012). After careful consideration (given the potential serious consequences of this side effect), trials have now restarted. We consider potential side effects of anti-NGF therapy in the following section.

7. POTENTIAL SIDE EFFECTS OF ANTI-NGF ANALGESIC THERAPY AND HOW TO MITIGATE THEM

Reports of rare cases of rapidly progressive joint degeneration necessitating joint replacement led to the FDA to halt all anti-NGF trials. This most commonly affected the hip and knee joints. Detailed assessment by an independent adjudication committee reported that the vast majority of cases were due to rapidly progressive osteoarthritis rather than osteonecrosis (Hochberg 2015). Rapidly progressive osteoarthritis was felt to be a safety signal that occurred in a dose-dependent manner and was also associated with concomitant use of NSAIDs. The mechanism of this side effect is not known. One hypothesis is that it is due to a form of interaction between bone health, denervation, and reduced pain perception, a mechanism reminiscent of the Charcot joint—a known consequence of neuropathy (Chisholm & Gilchrist 2011). Certainly,

during development, trkA-positive peripheral afferents are apparently crucial for healthy femoral bone formation (Tomlinson et al. 2016). However, as described in detail below, although pain is reduced in response to anti-NGF therapy, there is no evidence of a loss of small fibers. An alternative suggestion is that the combination of NSAIDs and anti-NGF could impede bone healing (Harder & An 2003). After extensive discussion with the FDA, the hold was lifted in March 2015, and the FDA stipulated careful radiographic screening and monitoring of the autonomic nervous system (see below) as well as avoidance of concomitant use of anti-NGF therapy and NSAIDs.

An additional, longstanding safety concern is whether there is an ongoing requirement for the growth-promoting effects of NGF on adult nociceptive afferents and sympathetic neurons, which would be impaired by anti-NGF therapy. The aim of anti-NGF therapy should be to normalize NGF levels and signaling and not to eliminate NGF completely, given its potential role in adulthood. As discussed above, adult sensory neurons no longer require NGF for survival, although they remain responsive to NGF, which prominently enhances the outgrowth of sensory axons in vitro (Lindsay 1988). Reassuringly, in trials in which peripheral nerve function has been assessed explicitly using objective measures such as autonomic testing, intraepidermal nerve fiber density, and nerve conduction studies, no detrimental effect was observed of dosing up to 24 weeks duration. However, long-term follow-up studies will be required (Brown et al. 2014), especially in populations that may be particularly at risk, such as patients with preexisting neuropathy (Bramson et al. 2015).

Abnormal sensory phenomena have been reported ever since Phase I trials of anti-NGF and have been described in multiple patient populations in subsequent Phase II and III trials using different therapeutics. These sensory changes include burning sensation, paraesthesia, hypoesthesia, peripheral hyperesthesia, and sensory disturbance. Such sensory disturbance is most commonly reported a week or two after the first dose and is self-limiting. Interestingly, some of these positive sensory phenomena such as paraesthesia are mediated by fiber types (myelinated low-threshold afferents), which we would not normally expect to be NGF responsive. Given the lack of evidence for structural changes in sensory afferents, they may represent altered ion channel function. Further sensory symptoms described with anti-NGF therapy versus placebo are myalgia and arthralgia, and these may represent the immune response to a humanized monoclonal per se rather than representing specific effects of NGF binding.

The development of sympathetic neurons has long been known to be NGF dependent (Aloe et al. 1975). Toxicology studies of anti-NGF in nonhuman primates raised the possibility—which caused further concern in the FDA—that anti-NGF could affect the morphology of neurons within sympathetic ganglia. Preclinical studies have shown evidence of reversible shrinkage of sympathetic neurons in adulthood following anti-NGF treatment (Mullard 2015). However, this does not appear to have long-term consequences: 6-month anti-NGF dosing performed in nonhuman primates did not lead to any neuronal loss in sympathetic or sensory ganglia, and there was no physiological evidence of sympathetic dysfunction (Zorbas et al. 2011). In contrast, as would be predicted from the developmental role of NGF, prenatal dosing with anti-NGF monoclonals results in morphological abnormalities in both sympathetic and sensory ganglia (Bowman et al. 2015, Butt et al. 2014), and anti-NGF therapy should be avoided in women who are pregnant or considering pregnancy.

To summarize, some potential side effects of anti-NGF therapy that may have been expected from its biology, such as small fiber neuropathy, have not in fact been reported in reasonably large clinical trials. Obviously, ongoing vigilance is required, especially in long-term dosing. One serious adverse effect that occurs in a small subset of patients is rapidly progressive osteoarthritis. This was unexpected and may relate to a poorly understood interaction with NSAIDs. Because

most of the reported side effects of anti-NGF are dose related, effort will be needed to find the optimum therapeutic window.

8. CONCLUSION

Findings from the drug development literature confirm the difficulty of targeting multifaceted molecules such as NGF. However, given its promising analgesic efficacy and the high socioeconomic burden imposed by chronic pain, most of us in the field would likely recommend we persevere with NGF and its receptors as a future treatment. This is particularly true because several key elements regarding the preclinical expression patterns and biology of NGF in the context of nociception remain to be explored. There are outstanding questions as to the divergent intracellular consequences of NGF binding, the precise cell types involved in peripheral sensitization, and the downstream targets of NGF-induced transcription. A deeper and updated understanding of how NGF impacts sensory and sympathetic neurons is likely to significantly advance our chances of identifying the right molecules for further compound development.

DISCLOSURE STATEMENT

D.L.B. has acted as a consultant for Pfizer, TEVA Pharmaceutical Industries, Mundipharma, Lilly, GSK, and Abide Therapeutics in the past 3 years and was a member of EUROPAIN, a public-private IMI partnership between the European Union and EFPIA. The authors are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

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