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REVIEW

Nerve Growth Factor Signaling and Its Contribution to Pain

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Abstract: Nerve growth factor (NGF) is a neurotrophic protein essential for the growth, differentiation, and survival of sympathetic and sensory afferent neurons during development. A substantial body of evidence, based on both animal and human studies, demonstrates that NGF plays a pivotal role in modulation of nociception in adulthood. This has spurred development of a variety of novel analgesics that target the NGF signaling pathway. Here, we present a narrative review designed to summarize how NGF receptor activation and downstream signaling alters nociception through direct sensitization of nociceptors at the site of injury and changes in gene expression in the dorsal root ganglion that collectively increase nociceptive signaling from the periphery to the central nervous system. This review illustrates that NGF has a well-known and multifunctional role in nociceptive processing, although the precise signaling pathways downstream of NGF receptor activation that mediate nociception are complex and not completely understood. Additionally, much of the existing knowledge derives from studies performed in animal models and may not accurately represent the human condition. However, available data establish a role for NGF in the modulation of nociception through effects on the release of inflammatory mediators, nociceptive ion channel/receptor activity, nociceptive gene expression, and local neuronal sprouting. The role of NGF in nociception and the generation and/or maintenance of chronic pain has led to it becoming a novel and attractive target of pain therapeutics for the treatment of chronic pain conditions.

Keywords: nerve growth factor, nociception, sensitization, chronic pain

Introduction

Nerve growth factor (NGF) is a neurotrophic protein essential for the growth, differentiation, and survival of sympathetic and sensory afferent neurons during development.[1] NGF contributes to neuronal phenotype by modulating axonal guidance, gene transcription, neurotransmitter release, and synaptic plasticity.[2–4] In addition, NGF plays a pivotal role in the modulation of nociception in adulthood.[5,6]

This review highlights how NGF receptor activation and subsequent downstream signaling alter nociception. Specifically, we discuss how NGF can (i) in a short time frame (typically within minutes) lead to direct sensitization of nociceptors via actions at the site of injury, and (ii) in a longer time frame (several hours to days) change gene expression and render nociceptors more responsive via actions in the dorsal root ganglion (DRG). These actions contribute to anatomic remodeling that results in a wider nociceptor input from injured tissue and increases the nociceptive signaling from the periphery to the central nervous system (CNS), providing a rationale for future study of novel analgesics that neutralize NGF or antagonizes its receptors.

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Methods

This narrative review was intended to provide an overview of the effects of NGF on nociceptive signaling. Due to the broad scope of the review, and the substantial body of published literature, a narrative approach was utilized. The review was based on searches of PubMed and the authors' familiarity with the published literature. Search terms included concepts related to NGF and pain or nociception. Results included both animal and human studies. Recent publications were prioritized, though older pivotal studies were also included.

Results

Overview of NGF and Its Receptors

NGF (Figure 1) is a member of the neurotrophin family, which in mammals also includes brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5.[7] NGF is initially translated as a precursor, proNGF, which can be (i) cleaved intracellularly into mature β-NGF by furin, (ii) cleaved extracellularly by plasmin or matrix metalloproteinases, or (iii) remain intact and signal in its proNGF precursor form.[8–10] Inhibiting the processing of proNGF abolishes regulated secretion of the resulting mature NGF product.[11]

There are 2 receptors for NGF, p75 neurotrophin receptor (p75NTR) and tropomyosin receptor kinase A (TrkA).[12] TrkA has a higher affinity for mature NGF than for proNGF and activates neurotrophic signaling.[9,13] P75NTR has a higher affinity for proNGF and can activate both neurotrophic and apoptotic signaling, the later in the presence of sortilin.[8,14] There is an intricate functional relationship between the 2 NGF receptors, and the signaling outputs of NGF and proNGF (survival versus apoptosis) depend on the cellular context and the ratio of TrkA to p75NTR.[13]

TrkA is expressed in nociceptive sensory neurons and is thought to mediate most of the important effects of NGF on the nociceptive system.[6,23] In rats, about 40% of DRG sensory neurons express TrkA, including peptidergic fibers that innervate bone, skin, muscle, and viscera.[6,23] Following the release of NGF, which frequently occurs at sites of peripheral tissue injury, NGF can bind TrkA receptors located at peripheral nociceptor terminals. Upon binding of NGF to the extracellular region of TrkA, the receptor dimerizes, autophosphorylates, and initiates signaling events by docking and phosphorylating downstream targets.[24–26] The NGF-TrkA complex is internalized into endosomes where it can be retrogradely transported, recycled, or degraded.[26] Immediate pronociceptive effects resulting from NGF/TrkA signaling (such as modulation of ion channel activity) occur in the peripheral nociceptor terminal, while longer-term effects (such as modification of gene expression) occur in the soma following retrograde axonal transport of the NGF/TrkA complex to the DRG.[5,6] Three major signaling cascades initiated by TrkA activation include the phospholipase C-γ (PLCγ) pathway, the mitogen-activated protein kinase (MAPK)/Erk pathway, and the phosphoinositide 3-kinase (PI3K) pathway.[26]

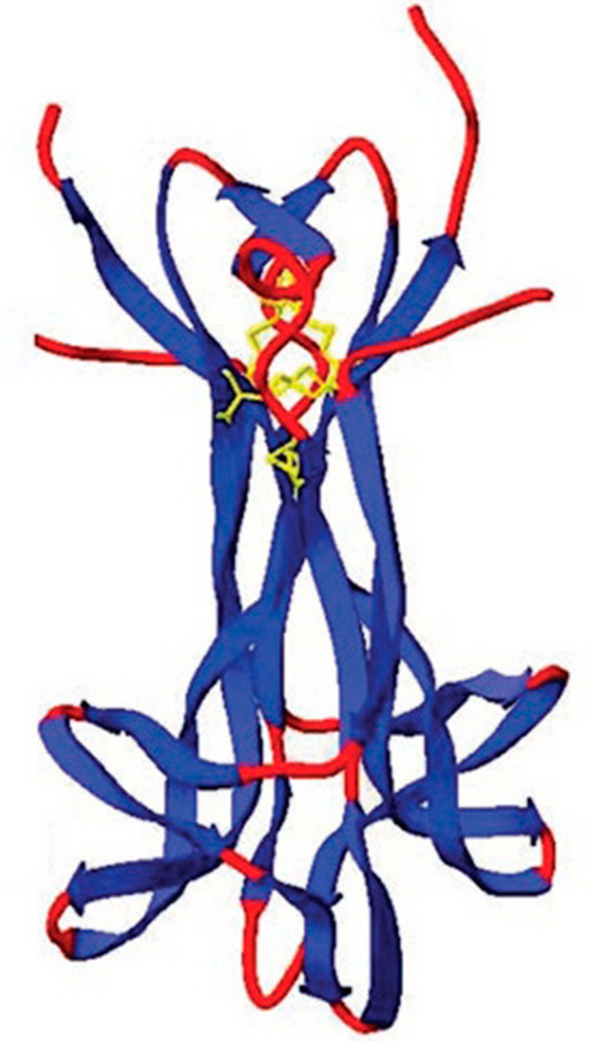

Figure 1 X-ray crystallographic structure of human NGF homodimer. NGF is a homodimer consisting of 2 strands of 120 amino acids each, which noncovalently dimerize to form a 26-kDa protein. Note the N-terminus of the monomers is not apparent (unresolved). Copyright© 2006. Portland Press. Reproduced with permission from Allen SJ, Dawbarn D. Clinical relevance of the neurotrophins and their receptors. *Clin Sci (Lond)*. 2006;110(2):175–191.[7]
Abbreviation: NGF, nerve growth factor.

Role of NGF on the Nociceptive System During Development

A dominant effect of NGF during early development is its role as a survival factor for neurons, including sympathetic and sensory neurons.[1,27] The density of the innervation of the target tissue is controlled by a spatially and temporally limited supply of NGF, and cells receiving insufficient support during this critical period of time succumb to cell death.[28]

NGF null mice have a severe loss of sympathetic and sensory neurons, particularly in the population of peptidergic small- and medium-diameter DRG neurons.[30] Animals lacking TrkA receptors show a phenotype similar to NGF null mice, underscoring the importance of NGF-TrkA signaling for the development of the nociceptive system.[30,31]

In humans, Hereditary Sensory and Autonomic Neuropathy type V (HSAN V) is caused by mutations in the NGF gene.[32,33] The first mutation identified, a cytosine to thymine point mutation at nucleotide 661, came from analysis of a northern Swedish multi-generational family.[32,34] This particular NGF mutation results in a substitution of tryptophan (W) for arginine (R) at amino acid 221 in proNGF (R221W), which corresponds to amino acid 100 in mature NGF (R100W).[35] This mutation causes a substantial loss of unmyelinated nerve fibers and a moderate loss of thinly myelinated fibers.[32] Patients with this mutation present with impaired ability to sense deep pain (pain originating in the bones or joints) and temperature (thresholds for heat and cold sensing are increased), but most other neurological functions, including sweating, appear normal.[32] This mutation does not affect NGF binding to TrkA but does reduce PLC signaling downstream of TrkA.[35] This NGF mutation also inhibits processing of proNGF to mature NGF, which may lower systemic NGF levels, and abolishes NGF binding to p75NTR.[34,35] Other mutations can alter the spectrum of HSAN V presentation. For example, a cytosine to adenosine mutation at nucleotide 680 (C680A) causes complete insensitivity to pain accompanied by anhidrosis, mild mental retardation, and immune deficiency.[33] Thus, different HSAN V NGF gene mutations may have a variety of effects on NGF-sensitive tissues.

Mutations in the TrkA gene cause a related disorder, HSAN IV, which produces a phenotype similar to HSAN V.[36] These TrkA gene mutations result in defective binding of NGF to TrkA and, as a result, the inhibition of NGF-induced TrkA phosphorylation and downstream signaling cascades.[37]

As development proceeds, the role of NGF in neuronal growth/survival during development diminishes and its role in modulating nociception becomes more relevant.[6] It is likely that the developmental role of NGF and the nociceptive role of NGF overlap temporally. The ability of NGF to modulate nociceptive signaling has been observed during early perinatal stages, with repeated postnatal (P0-14) exposure to exogenous NGF in rodents producing mechanical hyperalgesia that persists into adulthood.[38] Further, the ability of NGF to sensitize sensory neurons to capsaicin or heat stimuli begins between postnatal days 4 to 10.[39]

Evidence for a Role of NGF Signaling in Nociception in Adulthood

NGF Levels are Increased During Pain Conditions

Though adult sensory and sympathetic neurons can survive in the absence of NGF, NGF remains capable of promoting neuronal growth and sprouting in adulthood.[40–43] Basal NGF levels are lower in the adult than in development.[42,44] In humans, serum NGF levels start to decrease at approximately 8 years of age, presumably reflecting increasing maturity of the nervous system.[45] Levels of NGF increase in adult rodents in several inflammatory conditions and in several models of pain.[46–49] Further, blockage of NGF signaling can attenuate pain-related behavior in a variety of animal models including immune arthritis, fracture, bone cancer pain, osteoarthritis, and neuropathic pain.[50–60] Increased levels of NGF are also found in chronic pain conditions in humans, such as osteoarthritis, low back pain, and interstitial cystitis (Table 1).[61–77] However, an elevated level of NGF is not a hallmark of all chronic pain conditions and low levels of NGF have been found in the plasma of patients with fibromyalgia.[78] Thus, care should be taken when generalizing findings from one condition to another. It should also be noted that the physiologically relevant level of NGF required for neuronal sensitization at local sites of peripheral tissue injury is not known. It is also unclear how NGF levels at local sites of peripheral injury are correlated to overall levels measured in serum or other fluids.

NGF Administration Induces Hyperalgesia

In addition to the observation of increased NGF levels in chronic pain conditions and animal models of pain/inflammation, it has been demonstrated that exogenous

Table 1 Summary of Disease States or Conditions in Humans in Which Increased Levels of NGF Were Detected Compared with Controls

Study	Disease/Condition	Sample Size	Sample Matrix	NGF Form
Aloe et al[61]	Rheumatoid arthritis, osteoarthritis, or other chronic arthritis	n = 6 osteoarthritis patients; n = 8 rheumatoid arthritis patients; n = 8 patients with other chronic arthritis; n = 2 control patients who did not have rheumatic disease	Synovial fluid	Protein
Halliday et al[62]	Rheumatoid arthritis or other inflammatory arthropathy	n = 13 rheumatoid arthritis patients; n = 10 other inflammatory arthropathies; n = 3 normal volunteers	Synovial fluid	Protein
Walsh et al[63]	Rheumatoid arthritis or osteoarthritis	n = 10 rheumatoid arthritis patients; n= 11 osteoarthritis patients; n = 11 non-arthritic post-mortem controls	Vascular channels of osteochondral junction	Protein
Iannone et al[13]	Osteoarthritis	n = 12 osteoarthritis patients; n = 3 healthy controls	Knee chondrocytes	Protein
Jiang et al[65]	Interstitial cystitis/bladder pain syndrome	n = 30 interstitial cystitis/bladder pain syndrome patients; n = 26 controls	Blood serum	Protein
Okragly et al[66]	Interstitial cystitis or bladder cancer	n = 4 interstitial cystitis patients; n = 6 bladder transition cell cancer-carcinoma patients; n = 7 urinary tract infection patients; n = 7 healthy volunteers	Urine	Protein
Liu et al[67]	Interstitial cystitis/bladder pain syndrome	n = 58 interstitial cystitis/bladder pain syndrome patients; n = 28 healthy controls	Urine	Protein
Lowe et al[68]	Idiopathic sensory urgency, chronic cystitis, or interstitial cystitis	n = 4 patients with idiopathic sensory urgency; n = 4 chronic cystitis patients; n = 4 interstitial cystitis patients; n = 4 controls (genuine stress incontinence on cystometry but with no irritative symptoms)	Urothelium	Protein
Watanabe et al[69]	Chronic prostatitis (CP) or chronic pelvic pain syndrome (CPPS)	n = 20 CP or CPPS patients; n = 4 healthy male controls with no history of genitourinary symptoms, instrumentation, or surgery	Expressed prostatic secretions	Protein
Giovenga et al[70]	Primary fibromyalgia syndrome	n = 34 fibromyalgia syndrome patients; n = 15 patients diagnosed with fibromyalgia in addition to another painful or inflammatory condition; n = 10 other (patients diagnosed with another painful or inflammatory condition, but not fibromyalgia); n = 35 healthy controls	Cerebrospinal fluid	Protein
Sarchielli et al[71]	Chronic daily headache	n = 20 chronic daily headache patients; n = 20 age-matched controls who underwent lumbar puncture for diagnostic purposes	Cerebrospinal fluid	Protein
Sobue et al[72]	Various neuropathies[a]	n = 54 neuropathy; n = 4 specimens with normal appearance of morphology and normal nerve conduction	Sural nerve segments	mRNA
Freemont et al[73]	Low back pain	n = 21 "pain level" (discography at these levels reproduced the patients' symptoms of low back pain and/or sciatica) intervertebral disc (IVD) specimens; n = 20 "non-pain level" (discography was either painless or induced sensations that were not described by the patient as mimicking their symptoms) IVD specimens. A total of 41 specimens were taken from 36 patients	Intervertebral disc	mRNA

(*Continued*)

Table 1 (Continued).

Study	Disease/Condition	Sample Size	Sample Matrix	NGF Form
Richardson et al[74]	Low back pain	n = 5 samples from 4 non-degenerate post-mortem nucleus pulposus (NP) patients; n = 9 post-mortem degenerate NP samples from 4 patients; n = 13 surgical degenerate NP samples from 11 patients	Nucleus pulposus	mRNA
Aoki et al[75]	Lumbar degenerative disc disease	n = 29 patients with herniated discs; n = 26 patients with other degenerated disc diseases[b]	Nucleus pulposus	Protein
Zhu et al[76]	Pancreatic cancer	n = 37 pancreatic cancer patients; n = 27 pancreatic samples from humans free of pancreatic disease through an organ donor program in which there were no candidates for transplantation	Pancreatic cancer tissue[c]	mRNA

Notes: [a]Patients included had vasculitic and ischemic neuropathy; inflammatory demyelinating neuropathy with Guillain–Barre syndrome or chronic inflammatory demyelinating neuropathy; alcoholic neuropathy; familial amyloid polyneuropathy type 1; toxic neuropathy with cisplatin; Charcot–-Marie–Tooth disease type 1; X-linked recessive bulbospinal neuronopathy; diabetes mellitus; hypothyroidism; or neuropathy with unknown origin. [b]Other degenerated disc diseases were spondylolisthesis, spinal canal stenosis, and lumbar degenerative scoliosis. [c]Taken from patients undergoing a partial duodenopancreatectomy for pancreatic cancer.

administration or overexpression of NGF results in hyperalgesia and/or allodynia.[38,79-81]

Interestingly, striking hyperalgesic effects of NGF administration have also been observed in humans. In healthy adults, for example, a single subcutaneous injection of recombinant NGF has been shown to elicit local injection-site hyperalgesia that persists for up to 7 weeks, depending on the dosage.[82] Likewise, intradermal injection of NGF produces long-lasting local thermal (early onset) and mechanical (delayed) hyperalgesia.[83–87] Localized priming of nociceptors following intradermal injection of NGF has also been demonstrated through an enhancement of hyperalgesia in response to irradiation with ultraviolet-B.[88,89]

Intramuscular injection of NGF has been shown to cause lasting mechanical hyperalgesia in a variety of muscles.[90–101] Notably, injection of NGF into the tibialis anterior muscle induces local mechanical hyperalgesia within 3 hours of injection that spreads to distant areas on days 1 to 4, suggesting involvement of central pain mechanisms.[93] Repeated injections result in both temporal summation and spreading of mechanical pain, again implicating both peripheral and central mechanisms.[102] Spreading of NGF-induced hyperalgesia has also been observed following injection into the supraspinatus muscles.[94] A single injection of NGF into the facia of the musculus erector spinae muscle produces both mechanical and chemical (proton) hyperalgesia.[103] Chemical hyperalgesia has also been demonstrated following the injection of NGF into the tibialis anterior.[101]

NGF Treatment Lowers Nociceptor Activation Threshold

Intradermal injection of NGF increases the conduction velocity and decreases activity-dependent slowing of conduction velocity in unmyelinated porcine (pig) mechano-insensitive nociceptors.[104–106] The activation threshold of mechano-sensitive nociceptors at the injection site decreases following NGF treatment and the proportion of mechano-sensitive nociceptors increases.[104] While the receptive field of these nociceptors increased, there was no increase in intraepidermal nerve fiber density, suggesting that previously silent nociceptors may be recruited in this circumstance.[104] These changes were measured 3 weeks after NGF administration and, therefore, likely represent long-term effects of NGF signaling. Sensitization of skin nociceptors has been confirmed in humans using microneurography techniques which demonstrate that axonal branches exhibit reduced activation thresholds within the NGF injection zone but not outside of the injection zone.[107]

Nociceptive Actions of NGF Signaling

Effects of NGF on Inflammatory Cells and Mediators

There is evidence that NGF modulates nociception, in part, by influencing the actions of inflammatory cells and mediators. It has been shown that rodent mast cells produce and store NGF in granules until degranulation and NGF mRNA has been detected in a human mast cell line.[108,109] Moreover, cultured media from this mast cell line is able to induce neurite outgrowth in cultured chick embryonic sensory neurons, suggesting that NGF is secreted from these cells.[109]

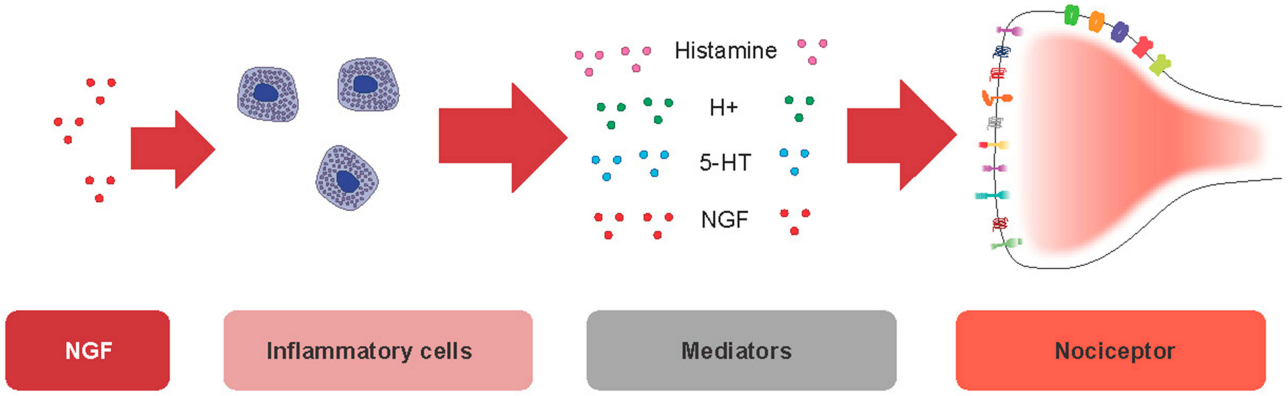

Figure 2 Nociceptive effects of NGF on inflammatory cells. NGF binds TrkA receptors on inflammatory cells. The resulting NGF/TrkA signaling increases the release of a variety of inflammatory mediators such as serotonin, histamine, and NGF itself, which are known to cause sensitization of nociceptors via modulation of receptor or ion channel activity at the peripheral terminal.
Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); NGF, nerve growth factor; TrkA, tropomyosin receptor kinase A.

NGF has also been found to be present in, and released from, human CD14+ T cell clones and human monocytes.[110,111]

NGF has been shown to increase the release of mediators from inflammatory cells (Figure 2). These mediators, such as bradykinin, histamine, ATP, serotonin, and protons, are released during inflammation or injury from ruptured cells or from infiltrating inflammatory cells and are capable of activating receptors and ion channels found on the peripheral nociceptor terminal, leading to neuronal depolarization and sensitization that manifests as pain hypersensitivity.[112] For example, exogenous IL-1β causes mechanical and thermal hyperalgesia (measured as an increased nociceptive reflex) in rodents, and histamine has been shown to mediate pain-related behaviors in a rodent model of interstitial cystitis.[113,114] Further, serotonin administered to healthy human volunteers causes mechanical hyperalgesia and stimulates calcium influx into cultured rat sensory neurons, an indication of cell excitability.[115,116] Finally, bradykinin treatment causes mechanical hyperalgesia in rats and Protein Kinase C (PKC) signaling-dependent sensitization of the transient receptor potential cation channel subfamily V member 1 (TRPV1), when isolated via patch-clamp, which has a known role in nociception and noxious heat sensation.[117,118]

NGF can trigger the release of histamine and leukotriene from human basophils, serotonin and histamine from rodent mast cells, and histamine and tryptase from a human mast cell line.[119–123] However, NGF administration did not activate mast cells in a separate rodent study, and there is some evidence that rodent mast cells do not express NGF receptors.[109,124] Though the contribution of mast cells to NGF signaling in humans is not clear, human mast cells express TrkA receptors and, thus, species differences must be considered when discussing the influence of NGF on inflammatory cells.[109] Similar to effects seen in mast cells, isolated murine peritoneal macrophages exposed to NGF increase the production of interleukin 1β (IL-1β).[125] This may occur through TrkA activation as TrkA expression, but not p75NTR expression, was observed in these cells.[125] The effects that NGF-mediated release of inflammatory mediators have will depend on the tissue. For example, histamine evokes the sensation of itch when released in isolation in superficial skin and mucous membranes, but causes burning pain when applied to deep somatic tissues.[126,127]

In addition to affecting cytokine release, NGF can also affect the actions of inflammatory mediators. For example, NGF can potentiate the sensitivity of rat DRG neurons to bradykinin.[128] On the other hand, inflammatory mediators can influence the levels and effects of NGF. Evidence suggests that IL-1β contributes to increased NGF levels in cultured sciatic nerve explants, and inhibiting bradykinin-1 receptor activity blocks NGF-induced thermal hyperalgesia in rodents.[114,129,130] Thus, there may be instances of positive feedback loops in vivo in which NGF stimulates the release and actions of inflammatory mediators that in turn stimulate increased synthesis and/or release of NGF. However, the role, if any, such a feedback loop plays in the generation or maintenance of chronic pain is not known.

NGF Effects on Nociceptive Ion Channels, Receptors, and Peptides

In addition to enhancing the release of inflammatory mediators that alter sensory neuron excitability, NGF signaling itself also has effects on the activity of nociceptive ion

Table 2 Summary of Short- and Intermediate/Long-Term Effects of NGF Signaling on Ion Channels, Receptors, and Peptides

Term	Effect	Downstream Signaling Pathways Possibly Involved
Short-term (typically within a few minutes)	• Increased TRPV1 channel activity.[131,134] • Increased P2X3 channel activity.[137] • Increased tetrodotoxin-resistant sodium channel activity.[140] • Decreased delayed rectifier potassium channel activity.[140] • Increased calcium channel activity.[202] • Increased NMDA receptor activity.[203]	• PLC/PKC, MAPK/ErK, Likely PI3K.[131–134] • PLC/PKC.[137] • Not identified • Not identified • Not identified • Possible direct interaction.[203]
Longer-term (typically several hours to days)	• Increased NaV1.8 synthesis.[204] • Increased NMDA receptor subtype 2B synthesis.[148] • Increased synthesis of TRPV1.[149] • Increased synthesis of voltage-gated calcium channels.[205] • Increased synthesis of P2X3.[153] • Increased BK2R synthesis.[128,154] • Increased activity of ASIC channels.[156] • Increased ASIC1a synthesis.[158] • Increased ASIC3 synthesis.[157] • Increased substance P synthesis.[159,160,174] • Increased CGRP synthesis.[47,159,174] • Increased BDNF synthesis.[179,206]	• Not identified • Not identified • Not identified • Not identified • Not identified • Not identified • Not identified • Not identified • NGF/TrkA and downstream PLC/PKC. NGF/p75NTR and downstream JNK/p38 MAPK.[157] • Not identified • Not identified • Not identified

Abbreviations: ASIC, acid-sensing ion channel; BDNF, brain-derived growth factor; BK2R, bradykinin receptor 2; CGRP, calcitonin gene-related peptide; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; P2X3, P2X purinoceptor 3; PI3K, phosphoinositide 3-kinase; PKC, protein kinase C; PLC, phospholipase C; TrkA, tropomyosin receptor kinase A; TRPV1, transient receptor potential cation channel subfamily V member 1.

channels and receptors that contribute to nociceptor sensitization (Table 2). The changes may be due either to direct, immediate effects on ion channel/receptor activity at the cell membrane and/or through longer-term effects such as enhanced gene transcription that leads to increased numbers of ion channels/receptors at the cell surface (Figure 3).

NGF Effects in Ion Channel Activity

The cation channel TRPV1, known to play a key role in nociception, is modulated by NGF activity. Cell culture studies have implicated each of the major signaling pathways downstream of TrkA activation in NGF-induced sensitization of TRPV1, though data particularly support a role for PI3K as a mediator of TRPV1 sensitization.[131–135] Another non-selective cation channel predominantly expressed in sensory neurons, the ATP-gated P2X3 receptor, is also modulated by NGF.[136–138] Cultured rodent trigeminal sensory neurons exposed to NGF exhibit potentiated P2X3 currents, while blocking NGF activity reduces such currents.[137,138] NGF-induced enhancement of P2X3 activity may occur downstream of TrkA activation, as PKC-mediated phosphorylation of P2X3 threonine subunits has been shown to increase P2X3 currents in these cultured neurons.[137,139]

In isolated rat primary DRG sensory neurons, NGF enhances tetrodotoxin-resistant sodium currents and suppresses delayed rectifier potassium currents, which together lead to increased cell excitability.[140] Signaling molecules downstream of TrkA activation have been shown to potentiate sodium channel activation. In cultured rodent DRG neurons, for example, Nav1.7 activation is increased via Erk1/2 signaling, and activation of p38 MAPK can directly phosphorylate Nav1.8 leading to an increase in Nav1.8 current density in DRG neurons.[141,142] However, whether these changes to sodium channel activation properties occur downstream of NGF-TrkA signaling, or as part of other signaling pathways, was not explored in these studies.

While numerous studies have demonstrated a role for NGF-TrkA signaling in the modulation of nociceptive ion channel activity, there is also evidence that NGF-p75NTR

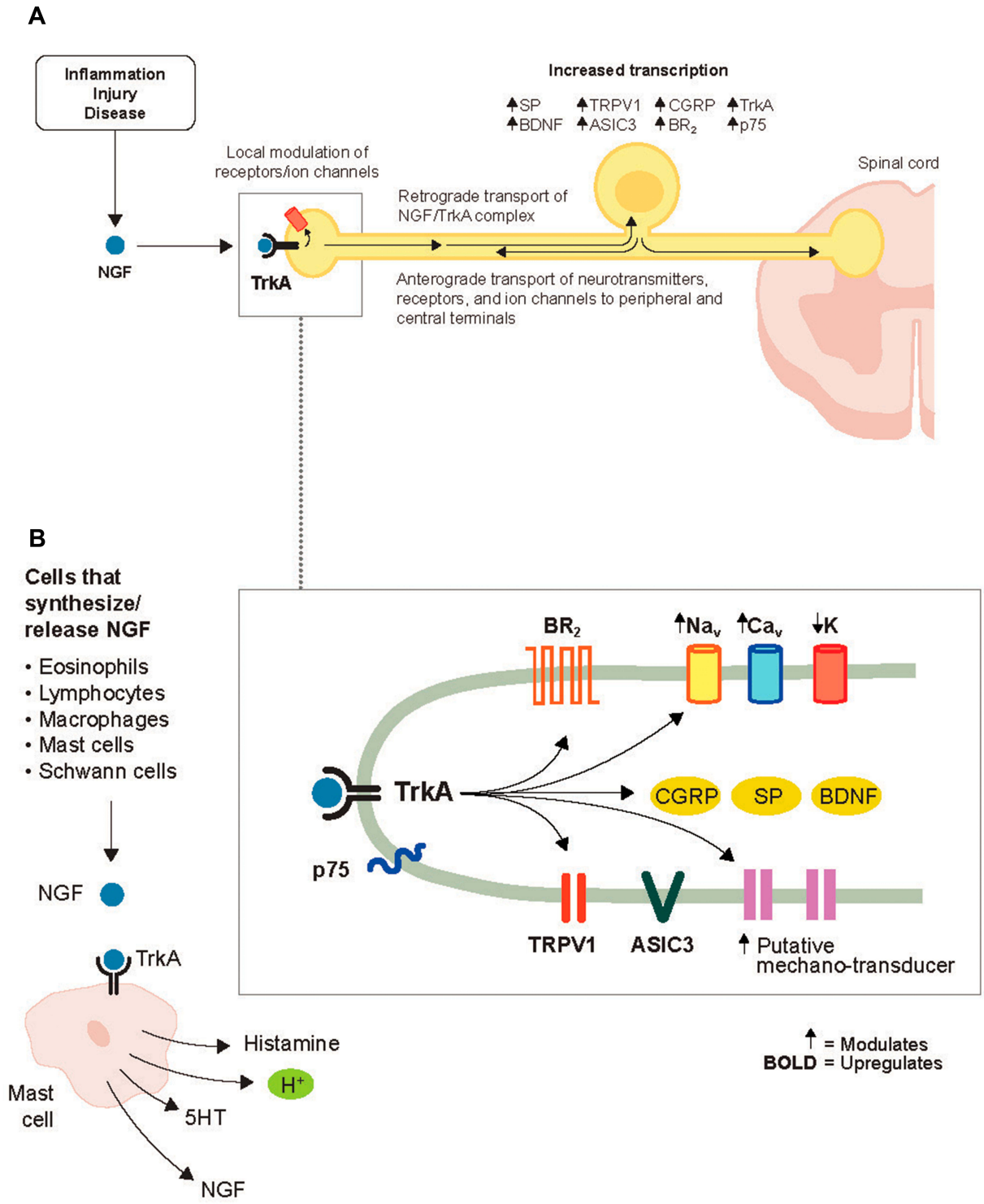

Figure 3 Effects of NGF on nociceptive ion channels, receptors, and peptides. (**A**) NGF signaling increases the activity of a variety of ion channels and receptors at the nociceptor peripheral terminal, which promotes depolarization and sensitization in a relatively short time frame. In a longer time frame, the NGF/TrkA complex is retrograde transported to the soma where NGF/TrkA signaling within the DRG promotes gene expression and leads to an upregulation of nociceptive ion channels, receptors, and peptides in the peripheral and central terminals. (**B**) NGF is released from a variety of cells following inflammatory injury. Reproduced with permission from Mantyh PW, Koltzenburg M, Mendell LM, Tive L, Shelton DL. Antagonism of nerve growth factor-TrkA signaling and the relief of pain. *Anesthesiology* (Official Journal of the American Society of Anesthesiologists). 2011;115(1):189–20[6]; https://anesthesiology.pubs.asahq.org/article.aspx?articleid=1933906.

Abbreviations: ASIC3, acid-sensing ion channel 3; BDNF, brain-derived neurotrophic factor; BR, bradykinin receptor; Ca, calcium; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; K, potassium; Na, sodium; NGF, nerve growth factor; SP, substance P; TrkA, tropomyosin receptor kinase A; TRPV1, transient receptor potential cation channel subfamily V member 1.

signaling can contribute to sensory neuron excitability.[6,143-145] For example, NGF-mediated activation of p75NTR has been shown to increase ceramide levels in a TrkA-independent manner in cell culture, and studies in rodents have shown that ceramide likely mediates NGF-induced sensitization of isolated sensory neurons in vitro and possibly NGF-induced pain-related behaviors in vivo.[140,146,147]

NGF Effects on Gene Expression

In addition to enhancing the activity of nociceptive ion channels to promote depolarization and sensitization in a short time frame, NGF also mediates longer-term changes in gene expression and/or membrane localization, both of which contribute to increased sensory neuron excitability. For example, intramuscular injection of NGF into the masseter of rats causes an increase in the number of trigeminal ganglion neurons expressing the N-methyl-D-aspartate (NMDA) receptor subtype 2B, an increase that peaks after 3 days and is associated with mechanical sensitization.[148] NGF has also been shown to promote TRPV1 transcription in PC12 cells and increase translocation of TRPV1 protein to the cell surface of cultured rodent DRG neurons, the latter possibly mediated through PI3K and/or PKC signaling events downstream of TrkA.[134,149–151] Increased expression of sodium channels is evident in DRG neurons, accompanied by behaviors associated with thermal and mechanical allodynia, after subcutaneous administration of NGF in rats.[152] Intrathecal administration of NGF in rats causes novel P2X3 expression in axons projecting to lamina I and outer lamina II of the spinal cord.[153] In freshly isolated mouse DRG, NGF exposure increases bradykinin B2 receptor mRNA and membrane expression.[154] Likewise, a separate study found that NGF treatment increases the number of bradykinin binding sites in these cells, which is dependent on the presence of p75NTR.[155]

Proton-gated acid-sensing ion channels (ASIC) levels may also be modulated by NGF. In cultured rodent DRG neurons, a mixture of inflammatory mediators including NGF, serotonin, interleukin-1, and bradykinin significantly increase ASIC3 currents, and NGF is known to increase ASIC3 expression.[156,157] In humans, local NGF-induced hyperalgesia in the tibialis anterior muscle is enhanced by subsequent treatment with acid, an activator of ASIC channels.[101] In this study, however, acute acid-induced pain was not enhanced by previous intramuscular injection of NGF.[101] This contrasts with a separate human study in which injection of NGF into the fascia of the Musculus erector spinae muscle enhanced painful responses to acidic saline treatment compared with control saline.[103] This difference may be due to the time required for retrograde transport of the NGF signaling complex to the DRG, since acid treatment occurred 7 and 14 days after NGF administration in the former study (enhanced acid response) and only 1 day after NGF administration in the latter study (no enhancement of acid response).[101,103] NGF signaling increases ASIC3 expression through a p75NTR-dependent transcriptional switch in primary cultured rat DRG neurons.[157] NGF controls a basal-level of ASIC3 transcription through constitutive activation of TrkA/PLC/PKC signaling, while increased levels of NGF promote ASIC overexpression via combined PLC/PKC and JNK/p38 MAPK signaling that depends on the presence of p75NTR.[157] ASIC1a protein expression has also been shown to increase following NGF treatment of cultured rat DRGs.[158]

Overall, the cellular processes mediating NGF-induced upregulation of ion channel membrane expression are not completely delineated and may involve a combination of effects on transcription, translation, and exocytosis.

NGF Effects on Peptides

NGF has also been shown to increase levels of peptides expressed by nociceptors including substance P and calcitonin gene-related peptide (CGRP), both of which are increased during inflammation.[47,159,160] NGF-mediated increases in substance P protein levels occur downstream of both TrkA and p75NTR activation in cultured rat sensory neurons.[160] While NGF's effects on nociceptive ion channels and cell surface receptors sensitize the nociceptor (more action potentials over time), NGF's ability to enhance neurotransmitter release (substance P and CGRP) potentially increases neurotransmission independent of increases in the number of action potentials. This synergistic effect makes NGF a novel therapeutic target relative to other known neuronal mediators such as bradykinin and serotonin.

NGF Effects on Nerve Sprouting

First-in-human studies using recombinant human NGF were designed to prevent or reverse peripheral neuropathy.[161] Phase 3 clinical trials not only failed to demonstrate a significant beneficial effect, but it was also observed that NGF injection produced generalized

myalgia and localized hyperalgesia at the injection site.[161] This observation revealed that intradermal NGF injections could be used as an experimental model for hyperalgesia and opened the door into research on how NGF modulates pain signaling. One thought was that local peripheral neuronal sprouting of sensory nerves can increase nerve terminal density in peripheral tissues. Such anatomical remodeling at sites of injury or inflammation could, potentially, contribute to increased nociceptive input and increased pain perception. For example, pathological sensory and/or sympathetic nerve sprouting, sometimes resulting in the formation of painful neuroma-like structures, has been observed in disease models of bone cancer pain and arthritis pain.[49,53,54,162] Evidence suggests that NGF can drive neuronal sprouting (Figure 4). For example, administration of NGF antibody inhibits sprouting and neuroma formation in the aforementioned models of bone and arthritis pain.[53–55,163]

In addition to its effect at peripheral sites, NGF may also play a role in neuronal sprouting at sites such as the DRG and dorsal horn of the spinal cord.[164–166] For example, axonal sprouting of peptidergic nociceptive neurons in the dorsal horn and into the ventral horn of the spinal cord can be induced by adenovirus-driven overexpression of NGF in rats.[165,166] Such sprouting leads to chronic pain, characterized by thermal-mechanical and hyperalgesia, in these animals.[165,166]

Although aberrant nerve sprouting has been seen in animal models of pain and evidence suggests this is NGF-dependent, the exact signaling pathways downstream of NGF receptor activation are unknown. Under in vitro experimental conditions, chick DRG axonal sprouting towards NGF-coated beads is blocked both by treatment with a pan-Trk inhibitor and with PI3K inhibition, consistent with the hypothesis that pathological sprouting may be mediated by NGF-TrkA signaling pathways.[167]

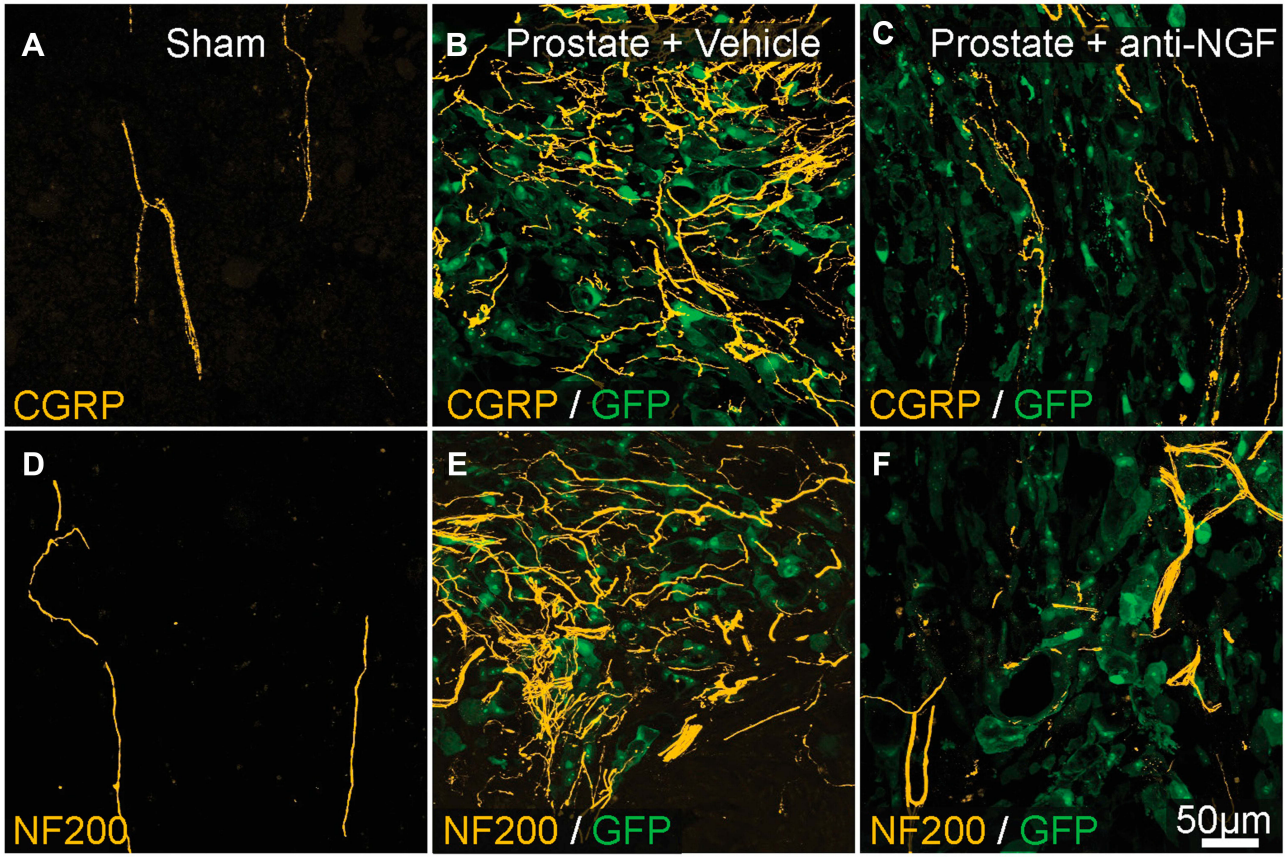

Figure 4 Preventative administration of anti-NGF antibody reduces metastatic prostate cancer-induced CGRP+ and NF200+ sensory nerve sprouting. (**A** and **D**) CGRP+ and NF200 + innervation of the bone marrow in sham-operated mice (yellow). (**B** and **E**) 26 days post-injection. Proliferation of prostate cancer cells (transfected with green fluorescent protein; green) and increased sprouting of CGRP+ and NF200+ fibers (yellow). (**C** and **F**) Effects of anti-NGF antibody (mAb911) administered at 10, 15, 20, and 25 days after cell injection. CGRP+ and NF200+ nerve sprouting has significantly reduced. Republished with permission from Pathological Sprouting of Adult Nociceptors in Chronic Prostate Cancer-Induced Bone Pain. Juan M. Jimenez-Andrade, Aaron P. Bloom, James I. Stake, William G. Mantyh, Reid N. Taylor, Katie T. Freeman, Joseph R. Ghilardi, Michael A. Kuskowski and Patrick W. Mantyh. *J Neurosci.* 2010;30 (44) :14649-14656.[163] https://doi.org/10.1523/JNEUROSCI.3300-10.2010.
Abbreviations: CGRP, calcitonin gene-related peptide; GFP, green fluorescent protein; NF200, 200-kDa neurofilament; NGF, nerve growth factor.

NGF also mediates sprouting of TrkA+ sympathetic nerve fibers.[168–171] Exogenous administration of NGF in adult mice, for example, leads to increased adrenergic nerve sprouting in several peripheral organs and in the brain.[168] An increase in sympathetic drive may represent another mechanism through which NGF contributes to pain. For example, increased sympathetic signaling plays a role in the maintenance of pain associated with complex regional pain syndrome (CPRS) and elevated sympathetic activity increases the spatial distribution of hyperalgesia in these patients.[172,173]

NGF Effects Within the CNS

As discussed above, NGF signaling contributes to acute and long-term nociceptive hypersensitivity by increasing the activity and/or expression of nociceptive ion channels, receptors, and peptides in the periphery. However, NGF may also have sensitizing effects within the CNS.

NGF has been shown to affect levels of nociceptive peptides within the CNS. Repeated subcutaneous administration of NGF increases CGRP and substance P release at central afferent terminals of sensory neurons in rodents.[174] CGRP increases neuronal excitability of spinal neurons and substance P has been shown to increase dorsal horn neuron excitability by potentiating NMDA activity in these animals.[175–177] NGF also affects BDNF levels, a neurotrophin that is expressed by some TrkA+ sensory neurons, and BDNF release in the spinal cord is thought to contribute to the central sensitization thought to underlie many chronic pain conditions.[178] In adult rats, BDNF mRNA levels are selectively increased in TrkA-expressing DRG cells in response to intrathecal administration of NGF.[179] Following NGF treatment, BDNF is retrogradely and anterogradely transported from the DRG to the peripheral and central sensory nerve terminals.[179,180] BDNF is also released directly in the dorsal horn following electrical stimulation of dorsal roots in isolated rat dorsal horns, and this release is enhanced by systemic or intrathecal NGF administration.[181] BDNF increases sensory neuron excitability via binding to p75NTR and subsequent downstream sphingosine kinase signaling.[182] BDNF can also sensitize rodent spinal lamina II neurons via NMDA receptor activation and PLC/PKC signaling, though it is not known whether the PLC/PKC signaling pathway is initiated downstream of TrkA activation in this case.[183]

NGF may also play a role in wind-up, the process by which central neuron excitability is increased following repeated low-frequency stimulation.[184] Isolated rat spinal cords treated with NGF exhibit a novel wind-up response with low-frequency stimulation of group I/II Aβ fibers that were found to be mediated through enhanced neurokinin-1 receptor activation.[185]

Overall, NGF signaling initiated at distal peripheral locations can have long-lasting effects within the CNS that may contribute to chronic pain (Figure 5). A single subcutaneous administration of NGF in the rat, for example, causes transient thermal and mechanical allodynia (up to 24 hours), but persistent (up to 3 months) increases in sodium channel levels within neurons of the DRG.[152]

Future Perspectives

Given the role of NGF in the modulation of nociception, the analgesic benefits of drugs targeting the NGF pathway have been explored in pre-clinical pain models and in human studies. Monoclonal antibodies against NGF (eg, tanezumab and fasinumab) that bind and neutralize NGF activity are in late stages of clinical development, having demonstrated significant analgesic effects over placebo in Phase 2 or Phase 3 trials of osteoarthritis.[186–196] Small molecule TrkA inhibitors (ASP7962 and GZ389988A) have advanced to Phase 2 clinical testing with mixed results. A single intra-articular injection of the TrkA inhibitor GZ389988A has been shown to modestly improve osteoarthritis knee pain at 8 weeks.[197] In contrast, treatment with the oral TrkA inhibitor ASP7962 at a dose of 100 mg BID failed to improve pain and function in patients with knee osteoarthritis after 4 weeks of treatment.[198] Finally, a Phase 1 trial of LEVI-04, an injectable p75NTR fusion protein designed to bind excess NGF, is currently recruiting healthy volunteers and patients with knee OA (NCT03227796).

Other novel pain therapeutics targeting the NGF pathway are in the early stages of discovery or pre-clinical development. These include monoclonal antibodies that bind and neutralize TrkA and small molecule NGF/proNGF inhibitors that disrupt NGF/proNGF binding to TrkA and p75NTR.[199–201] While still in early developmental stages, these small molecule-based inhibitors may be of therapeutic interest in attenuating NGF-induced sensitization of nociceptive signaling pathways.

The nociceptive signaling pathways mediated by NGF have been studied primarily in vitro in cell culture studies or in vivo using animal models. However, signaling pathways may differ in human cells. With advances in human induced pluripotent stem cells, it may be possible in the

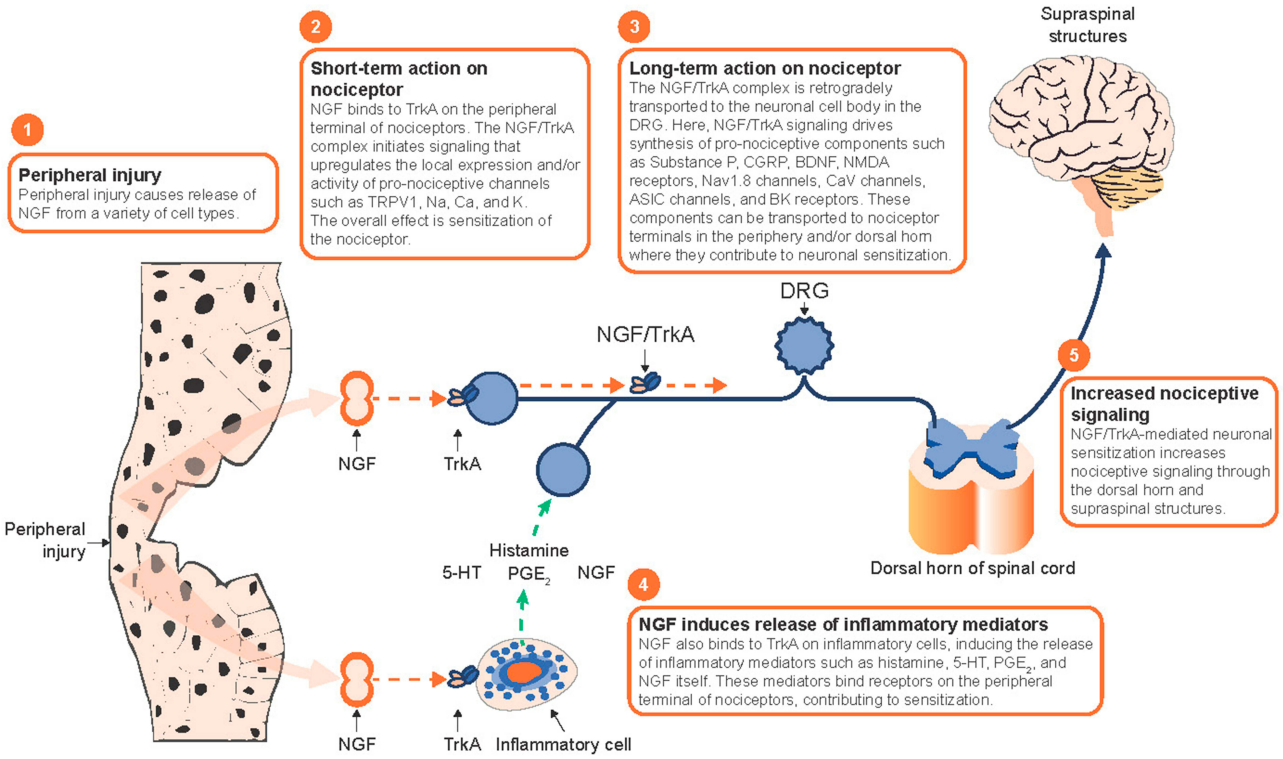

Figure 5 Summary of NGF effects on nociception. NGF/TrkA signaling has relatively short-term actions at the peripheral nociceptor terminal and on inflammatory cells, followed by longer-term actions within the nociceptor soma in the DRG. The overall effect is neuronal sensitization in the periphery and in the dorsal horn, leading to increased nociceptive signaling to higher-order pathways. Reproduced with permission from Schmelz et al. Nerve growth factor antibody for the treatment of osteoarthritis pain and chronic low-back pain: mechanism of action in the context of efficacy and safety. *Pain* (Official Journal of the International Association for the Study of Pain). 2019 Oct;160(10):2210–2220; https://journals.lww.com/pain/Fulltext/2019/10000/Nerve_growth_factor_antibody_for_the_treatment_of.6.aspx.[207]

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); ASIC, acid-sensing ion channels; BDNF, brain-derived neurotrophic factor; BK, bradykinin; Ca, calcium; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglion; K, potassium; Na, sodium; NGF, nerve growth factor; PGE_2, prostaglandin E_2; SubP, substance P; TrkA, tropomyosin receptor kinase A; TRPV1, transient receptor potential cation channel subfamily V member 1.

future to study NGF-induced nociceptive signaling pathways in sensory neuron-like cells derived from human pluripotent stem cells, allowing for a better understanding of the cellular role of NGF in human nociception.[171]

Conclusions

NGF has a well-known and multifunctional role in nociceptive processing; however, the precise signaling pathways downstream of NGF receptor activation that mediate nociception are complex and not completely understood. Additionally, much of the existing knowledge derives from studies performed in animal models, and this may not accurately represent the human condition. However, available data establish a role for NGF in the modulation of nociception through effects on the release of inflammatory mediators, nociceptive ion channel/receptor activity, nociceptive gene expression, and local neuronal sprouting. The role of NGF in nociception and the generation and/or maintenance of chronic pain have led it to become a novel and attractive target of pain therapeutics for the treatment of chronic pain conditions.

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Disclosure

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