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Title:

Nerve Growth Factor and the Physiology of Pain: Lessons from Congenital Insensitivity to Pain with Anhidrosis

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Conflict of interest

The author reports no conflict of interest.

Abstract

Congenital insensitivity to pain with anhidrosis (CIPA) is an autosomal recessive genetic disorder characterized by insensitivity to pain, anhidrosis (the inability to sweat) and mental retardation.

Nerve growth factor (NGF) is a well-known neurotrophic factor essential for the survival and maintenance of NGF-dependent neurons, including primary afferent neurons with thin fibers and sympathetic postganglionic neurons, during development. NGF is also considered to be an inflammatory mediator associated with pain, itch and inflammation in adults.

CIPA results from loss-of-function mutations in the *NTRK1* gene-encoding TrkA (tropomyosin-related kinase A), a receptor tyrosine kinase for NGF. Defects in NGF-TrkA signal transduction lead to the failure of survival of various NGF-dependent neurons. As a result, patients with CIPA lack NGF-dependent neurons. Recent studies have revealed that mutations in the *NGFB* gene-encoding NGF protein also cause congenital insensitivity to pain.

Using the pathophysiology of CIPA as a foundation, this review investigates the ways in which NGF-dependent neurons contribute to interoception, homeostasis and emotional responses and, together with the brain, immune and endocrine systems, play crucial roles in pain, itch and inflammation.

The NGF-TrkA system is essential for the establishment of neural networks for interoception, homeostasis and emotional responses. These networks mediate reciprocal communication between the brain and the body in humans.

Keywords:

congenital insensitivity to pain (CIP), congenital insensitivity to pain with anhidrosis (CIPA), hereditary sensory and autonomic neuropathy type IV, interoception, NGF-dependent primary afferent neurons, *NTRK1* gene, polymodal receptors, receptor tyrosine kinase for NGF, sympathetic neurons, TrkA receptor,

Introduction

Nerve growth factor (NGF) was the first growth factor to be identified and characterized (1). NGF supports the survival and maintenance of peripheral neurons derived from the neural crest and neurons in the brain during embryonic development and in the early postnatal stage. However, NGF also plays an important role in inflammatory processes in adult animals (2-4).

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Traditional animal studies of pain using electrophysiology, pharmacology and/or molecular biology have yielded valuable insights into the molecular basis of pain perception (5-7). Genetic approaches using gene-knockout mice have complemented these studies and contributed to the study of pain pathways (8, 9). Pain is a subjective and emotional experience, and it is often difficult to evaluate this aspect of pain in animal studies. Experimental studies of pain involving humans as subjects are usually not permitted due to ethical reasons.

Rare human genetic disorders provide opportunities to explore pathological conditions in humans and the underlying normal biological processes. In view of this, genetic variants leading to loss of pain have implications for pain medicine. Congenital insensitivity to pain (CIP) includes several genetic disorders associated with the inability to detect noxious stimuli. Patients with CIP lack pain sensation. The molecular pathophysiology of CIP can provide important clues about the biological processes of pain in our species and insights into the mechanisms that underlie persistent or chronic pain syndromes.

CIP is divided into two types: with anhidrosis (the inability to sweat) and without anhidrosis. Congenital insensitivity to pain with anhidrosis (CIPA) is the first human genetic disorder for which the molecular basis of CIP has been identified. CIPA is caused by loss-of-function mutations in the *NTRK1* gene-encoding TrkA (tropomyosin-related kinase A), a receptor tyrosine kinase for NGF (10). Recent studies have identified the genetic bases of other forms of CIP, including CIP due to defects in NGF itself (11-13) and channelopathy due to loss-of-function mutations in the *SCN9A* gene (14-16).

It is well-known that NGF has two receptors: the p75 neurotrophin receptor (p75^{NTR}), a member of the tumor necrosis factor receptor superfamily, and TrkA. p75^{NTR} is a low-affinity receptor for NGF and other neurotrophins, including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (17). p75^{NTR} is involved in various mechanisms of NGF-induced neural modulation. However, no

human genetic disorders attributable to loss-of-function mutations of p75^{NTR} have been described thus far. This review focuses on CIP due to genetic defects of NGF and TrkA. The pathophysiology of CIP might provide some clues to clarify the functions of the NGF-TrkA system in humans.

NGF and its receptor tyrosine kinase, TrkA

NGF is initially synthesized as a proform of NGF (proNGF) that is cleaved to release the mature active protein, NGF (17). The mature protein forms a stable non-covalent dimer that is normally expressed at very low levels during development. NGF mediates biological effects by binding to and activating TrkA receptors at nerve terminals (17). The activated TrkA receptors then stimulate local effects at nerve terminals and retrograde effects at neuronal cell bodies that often reside at considerable distances from the terminals. The major retrograde signal required for survival and gene expression consists of activated TrkA itself.

The *NTRK1* (also known as *TRKA*) gene was first detected and isolated in 1986 as a part of the *trk* oncogene derived from human colon carcinoma (18, 19). The physiological role of TrkA was then unveiled when TrkA was shown to be the signaling receptor for NGF (20, 21). Later, other members of the Trk family were discovered and characterized. TrkB is the signaling receptor for BDNF and NT-4, while TrkC is the primary receptor for NT-3 (22). NT-3 also binds to TrkA and TrkB, albeit with significantly lower affinity.

A single transmembrane domain divides TrkA proteins into extracellular and intracellular domains (Fig. 1) (22, 23). The extracellular domain is important for specific NGF binding and includes a signal peptide, three tandem leucine-rich motifs flanked by two cysteine clusters and two immunoglobulin-like domains (or motifs). The intracellular domain includes a juxtamembrane region, a tyrosine kinase domain and a very short carboxy-terminal tail. The intracellular domain is phosphorylated in response to NGF and is critical for intracellular signaling. Phosphorylated tyrosine residues in the TrkA cytoplasmic domain serve as anchors for binding downstream signaling molecules (22, 24). *In vitro* assessments have identified TrkA tyrosine residues - 490, 670, 674, 675 and 785 as autophosphorylation sites (25).

The binding of NGF to TrkA stimulates homodimer formation and tyrosine kinase activity (Fig. 1). TrkA controls three major signaling pathways (17); NGF thus regulates neuronal survival and apoptosis at several levels. NGF functions as a survival signal and suppresses a cellular suicide program (apoptosis), while deprivation of NGF activates

cell death (26). The phosphatidylinositol-3-kinase (PI3-K) pathway induces suppression of apoptotic proteins, while the mitogen-activated protein kinase pathway promotes neuronal differentiation, including neurite outgrowth. NGF binding to TrkA receptors also activates phospholipase C- γ (PLC- γ) signaling pathways. PLC- γ signaling contributes to NGF-mediated neural hypersensitivity (27). These signaling pathways probably contribute to the short-term modulations of neurons such as increased amount (28, 29) or phosphorylation (30) of ion channels in the plasma membrane and activation of other signaling pathways (31). They are also important for the regulation of gene expression (32, 33).

Congenital insensitivity to pain with anhidrosis

CIPA is an autosomal recessive genetic disorder that is characterized by insensitivity to pain, anhidrosis (the inability to sweat) and mental retardation. CIPA is also known as hereditary sensory and autonomic neuropathy type IV (HSAN-IV) (34). Patients with CIPA exhibit insensitivity to both superficial and deep painful stimuli. Visceral pain perception is also impaired. Touch, vibration and position senses are normal. Motor functions are normal, although repeated trauma can induce secondary dysfunction in the motor system. Sweating is important in order to maintain body temperature, especially in humans--because patients with CIPA do not sweat, they tend to develop hyperthermia in hot environments.

CIPA results from loss-of-function mutations in the *NTRK1* gene (10, 35-43). The *NTRK1* gene is located on chromosome 1q21-22. Examples of *NTRK1* mutations responsible for CIPA are shown in Fig. 2. Patients with CIPA have no functional TrkA proteins. NGF is a neurotrophic factor essential for the survival and maintenance of various types of neurons, including primary afferent neurons with thin fibers, autonomic sympathetic postganglionic neurons and possibly several types of neurons in the brain. Defects in NGF-TrkA signal transduction lead to the failure of survival of various NGF-dependent neurons since these neurons are not maintained due to apoptosis during development (44). As a result, patients with CIPA lack all NGF-dependent neurons.

NGF-dependent primary afferent neurons with thin fibers (NGF-dependent primary afferents) are defined as primary afferent neurons with small diameters and thinly myelinated A δ -fibers or unmyelinated C-fibers that depend on the NGF-TrkA system during development (Fig 3). The lack of pain and the presence of anhidrosis in CIPA are due to the absence of NGF-dependent primary afferents and sympathetic postganglionic neurons, respectively.

Patients with CIPA most likely lack some neurons in the brain, as these patients show mental retardation and characteristic behaviors. Patients with CIPA have mental retardation to variable degrees and exhibit learning deficits. Affected children show defects in conceptual thinking, abstract reasoning and social behavior and exhibit symptoms of moderate to severe emotional disturbance (45, 46). Hyperactivity and emotional lability are common. Their behavior of children with CIPA is often characterized as labile, hyperactive and erratic.

Conducting neuroanatomical studies of the forebrains of patients with CIPA would be intriguing, though no obvious gross abnormalities were recognized in one patient examined more than four decades ago (47). Corresponding gene-knockout mice lack basal forebrain cholinergic neurons (BFCNs) and striatal cholinergic neurons (9). Neither BFCNs nor striatal cholinergic neurons mature fully in knockout mice in the absence of NGF/TrkA signaling (48). Therefore, it is likely that patients with CIPA lack the corresponding human neurons.

BFCNs include neurons of the nucleus basalis of Meynert, the medial septal nucleus and the vertical and horizontal nuclei of the diagonal band (Broca) (49, 50). BFCNs are projection neurons, the axons of which extend throughout the hippocampus and the neocortex, and are important for learning, memory and, more specifically, processes of attention (49, 51). Striatal cholinergic neurons are large interneurons involved in the control of movement. Recent studies of rodents and humans suggest that the striatum is critical for the procedural memory involved in forming behavioral habits (52). The importance of cholinergic neurons and NGF signaling in neurodegenerative diseases is also well documented (53). Animal studies, as well as human studies, indicate the existence of other NGF-dependent neurons in the brain. Further studies are needed to determine the functional significance of these neurons.

Congenital insensitivity to pain due to mutations of the *NGFB* gene

Norrbottnian congenital insensitivity to pain

A missense mutation in the *NGFB* gene-encoding NGF protein has been detected in a large family from northern Sweden (11, 12, 54). In normal individuals, NGF is initially synthesized as the proNGF and proteolytically processed and secreted as mature NGF (17). The mutation in this family substitutes C to T at nucleotide position 661 and changes a basic Arg to a non-polar Trp at position 211 (R221W) in proNGF (or position 100 in the mature NGF protein). Expression studies of this mutant protein show

markedly reduced secretion of mature form; protein bearing this substitution is found mainly in the form of proNGF (55, 56). Therefore, decreases in the availability of mutant mature NGF and losses of trophic support of NGF-dependent neurons are most likely responsible for the phenotype of CIP observed in this family.

Homozygous patients have a severe congenital form of the disease with onset of symptoms occurring at an early age and most often affecting the lower extremities with insidious progressive joint swelling or painless fractures (54). Arthropathy tends to be progressive, resulting in disabling Charcot joints with gross deformities and instability. These patients lack deep pain perception in bones and joints and have no protective reflexes, which lead to gross bone and joint complications. They also show impaired temperature perception with normal sweating. No mental retardation is observed. In contrast, heterozygous patients have mild or moderate problems with joint deformities, usually with only slight discomfort (54). Some heterozygous patients are without symptoms.

Sural nerve biopsies from homozygous patients show severe reductions in the numbers of unmyelinated C- fibers and moderate losses of thin myelinated A δ fibers (54). Nerve biopsies from heterozygous patients show moderate losses of both A δ and C- fibers. Interestingly, the same results are seen in either symptom-free or neuropathic heterozygous subjects.

Therefore, the phenotype of CIP in this family is less severe than that of patients with CIPA. The inheritance pattern of CIP in this family seems to be autosomal dominant since either homozygous or heterozygous individuals show insensitivity to pain to varying degrees. Individuals who are heterozygous for the NGF mutation in this family show a milder phenotype than homozygous individuals. This suggests that either there is a gene dosage effect for the R221W mutation (12, 56) or the mutation might have a small dominant-negative effect in heterozygotes (13) –for instance, low levels of secreted R221W proteins may interfere with wild-type NGF homodimerization, thus resulting in reduced NGF function.

A loss-of-function mutation in the *NGFB* gene

A homozygous loss-of-function has been detected in a consanguineous Arab family in which five of the six children are completely unable to perceive pain (13). This mutation, c.[680C>A] + [681_682delGG], is a C to A transversion at nucleotide 680 and a two base deletion at nucleotide 681-681, causing a frame-shift at amino acid valine 232 and the replacement of 15 amino acids with a novel 43 amino acid terminal

sequence (V232fs). The mutant gene produces precursor proteins V232fs that have no biological activities.

Homozygous patients in this family are completely unable to perceive pain and are mentally retarded. They do not discriminate between temperatures nor do they sweat. Consanguineous, heterozygous parents have no clinical phenotype, do not report high pain thresholds and have not suffered from any painless injuries or particular medical problems. The absence of phenotype in the parents indicates autosomal recessive inheritance of CIP in this family. Sural nerve biopsies were not available.

The phenotype of the patients was categorized as hereditary sensory and autonomic neuropathy type V (HSAN-V) in the report (13). However, the classification of HSAN-V is controversial (57, 58). The characteristic phenotypes observed in patients with CIP from this family are similar to those observed in patients with CIPA. This suggests that CIPA can be caused by loss-of-function mutations in the *NGFB* gene.

It is interesting to note that why CIPA in this Arab family includes mental retardation and anhidrosis and Norrbottnian CIP does not. The reason might be attributed to a complete defect of NGF protein in this family, compared with a partial defect of the same protein in Norrbottnian CIP, sparing mental activity and sweating. Further studies are needed to clarify this.

Anatomy and functions of NGF-dependent neurons: lessons from CIPA

Interoception and polymodal receptors

NGF-dependent primary afferents innervate all tissues of the body, including the skin, muscles, joints, teeth and viscera, and have small diameters and consist of thinly myelinated A δ -fibers or unmyelinated C-fibers (Fig. 3). The cell bodies of NGF-dependent primary afferents are located in the dorsal root ganglia alongside the spinal cord for the body or the trigeminal ganglia for the face and have peripheral and central axonal branches that innervate their target organs and spinal cord, respectively. A subset of neurons in the glossopharyngeal nerve (IX) and the vagus nerve (X) are most likely NGF-dependent primary afferents because visceral pain perception is impaired in patients with CIPA. These afferent neurons in the IX and X transmit visceral afferent information to the brain from the head and neck and from the thoracic and abdominal cavities, respectively.

NGF-dependent primary afferents not only detect noxious stimuli, but also transmit sensations from the body's interior, the so-called interoceptive sense (59, 60). The

interoceptive system is considered to be a homeostatic afferent pathway representing the physiological status of all tissues of the body, including the mechanical, thermal, chemical, metabolic and hormonal status of the skin, muscles, joints, teeth and viscera. NGF-dependent primary afferents are thus also referred to as ‘interoceptive polymodal receptors’ (61, 62). Interoceptive polymodal receptors show slow activities that transmit changes in a wide variety of physiological conditions, such as temperature, mechanical stress, local metabolism (acidic pH, hypoxia, hypercapnea, hypoglycemia, hypo-osmolarity and lactic acid), cell rupture (ATP and glutamate), cutaneous parasite penetration (histamine), mast cell activation (serotonin, bradykinin and eicosanoids) and immune and hormonal activity (cytokines and somatostatin) (59, 60). Therefore, NGF-dependent primary afferents, i.e. interoceptive polymodal receptors - comprise both homeostatic afferent pathway and nociceptive pathway.

Sympathetic postganglionic neurons

Sympathetic postganglionic neurons are also NGF-dependent neurons and innervate blood vessels, piloerector muscles and sweat glands in the skin as well as other target organs and tissues in the body (Fig. 3). Sympathetic neurons regulate sweating in hot environments and piloerection and vasoconstriction in cold environments. Therefore, these neurons play critical roles in the homeostasis of body temperature, especially in humans. Because patients with CIPA do not sweat, they tend to develop hyperthermia in hot environments and suffer from hypothermia in cold environments. Clinical and behavioral studies also suggest that patients with CIPA lack sympathetic regulation of various target tissues, including internal organs.

NGF-dependent neurons contribute to pain, itch and inflammation

Injury or tissue damage activating NGF-dependent primary afferents causes the sensation of pain (Fig. 3) and activates sympathetic responses. These responses are involved in various reactions that protect the body, including withdrawal reflexes and vasoconstriction. Upon stimulation, NGF-dependent primary afferents release various neuropeptides [(e.g. substance P (SP) and calcitonin gene-related peptide (CGRP)) that modulate inflammation, pain and pruritus (Fig. 3). In turn, these neuropeptides trigger the release of pro-inflammatory mediators that amplify or facilitate inflammation by enhancing vasodilation, blood flow, vascular leakiness and leukocyte trafficking to sites of inflammation (63).

Insect bites and invasion by parasites also activate NGF-dependent primary afferents and cause itch sensations that create the desire to scratch the skin. Injury and microbial invasion stimulate the local release of numerous chemicals that mediate or facilitate inflammatory processes (64, 65) and influence the expression of NGF. Therefore, both pain and itch sensations are related to local tissue damage and inflammatory responses.

Autonomic sympathetic nerves innervate various cells in the body and thereby maintain homeostasis and regulate inflammation and host defenses. Therefore, mediators derived from NGF-dependent primary afferents or peripheral autonomic neurons, including sympathetic postganglionic neurons, play important regulatory roles in the body under many physiological and pathological conditions (62). Pain and itch sensations also provoke emotional responses in the brain and contribute to the creation of memories surrounding tissue-damaging events. It is likely that patients with CIPA lack these neural processes.

NGF-dependent neurons and the brain, immune and endocrine systems

The nervous, immune and endocrine ‘super-systems’ engage in multiple interactions in response to acute and chronic stress (66, 67). The brain is the central organ of stress, and the brain and immune system are essential for homeostatic regulation and survival (66). The endocrine system is engaged in coordinating and controlling complex responses of the brain and immune system (63, 66-68).

NGF-dependent neurons play critical roles in mediating cross-talk among these three ‘super-systems’ (Fig. 4). NGF-dependent primary afferents detect and transmit local changes associated with injury, tissue damage and/or associated inflammatory or immunological reactions. These reactions include those involved in the innate immune system (63, 65, 69). The systemic response of the sympathetic nervous system to dangerous signals, including internal events such as pain or external events such as threats, is also well-known as the ‘fight-or-flight’ response. Interestingly, NGF-dependent sympathetic postganglionic neurons constitute a branch of the efferent sympathetic system (Fig. 4). Indeed, patients with CIPA show the absence of sympathetic skin responses (70) and low levels of plasma norepinephrine after thirst tests or during surgical procedures (71). These results indicate that no sympathetic reactions occur during stressful events in patients with CIPA.

NGF-dependent primary afferents or sympathetic postganglionic neurons influence inflammation by secreting pro-inflammatory or anti-inflammatory substances into sites of inflammation (63, 65-69, 72, 73). The term ‘neurogenic inflammation’ refers to signs

of inflammation (e.g. tumor, rubor, calor and dolor) that develop upon the activation of neurons and the consecutive release of a neuronal mediator (74). The axon reflex is an efferent function of the NGF-dependent primary afferents (Fig. 3). Indeed, patients with CIPA lack the axon reflex responsible for neurogenic inflammation. This suggests that neurogenic inflammation does not occur without NGF-dependent neurons.

It would be worthwhile to mention here the importance of mast cells in the brain, immune and endocrine systems (Fig. 4). The mast cell is a resident cell in various tissues and is a critical effector cell for inflammation. Mast cells are located perivascularly, close to NGF-dependent neurons containing neuropeptides such as SP and CGRP (67-69). In response to injury or tissue damage, bradykinin, a peptide that stimulates these neurons, is produced, causing pain sensations (4). Mast cells also release various inflammatory mediators, including histamine, serotonin and eicosanoids in response to bradykinin. Various inflammatory mediators derived from mast cells induce inflammation and also stimulate NGF-dependent neurons.

Mast cells can contribute to multiple features of acute, chronic and allergic inflammation (69, 75). In allergic inflammation, inflammatory mediators derived from mast cells stimulate NGF-dependent primary afferents of the nose, skin and airways, resulting in sneezing, itching and coughing (75). Therefore, mast cells are ideally equipped and placed to integrate and relay signals from all three super-systems during peripheral tissue responses to psychological or pathological stress (67, 69).

NGF acts as a peripheral pain mediator, particularly during inflammatory pain states, in adults (2, 4). The expression of NGF is high in injured and inflamed tissues and is upregulated in a wide variety of inflammatory conditions (2, 76, 77). NGF is an important component of the chemical milieu of inflammation (7). The activation of TrkA receptors on NGF-dependent neurons triggers and potentiates pain signaling through multiple mechanisms (78). The sensitization of NGF-dependent neurons by inflammation or NGF contributes to the development of hypersensitivity in neighboring tissues.

Patients with CIPA lack NGF-dependent neurons and therefore they probably cannot mediate various neural or inflammatory processes via these neurons in the brain, immune and endocrine systems. Again, these patients are at a disadvantage, as the lack of critical neural and inflammatory processes threatens survival. In accordance with the concept of 'super-systems,' NGF-dependent neurons are considered to be communication routes between the brain, immune and endocrine systems. The NGF-TrkA system thus contributes to the establishment of neural networks in 'super-systems.'

NGF-dependent neurons and emotional responses

NGF-dependent neurons, i.e. primary afferents and autonomic sympathetic postganglionic neurons, form an interface between the nervous system and the body-proper (Fig. 5). Most information from the body is conducted to the brain unconsciously and the brain maintains homeostasis in the body via feedback mechanisms for which autonomic sympathetic neurons are indispensable. This is well illustrated by (unconscious) homeostatic control of body temperature.

In humans, systemic responses of the sympathetic nervous system often accompany emotional responses. When humans are exposed to danger or trauma, stimuli or contexts associated with the danger or trauma become learned triggers that unleash emotional responses (79, 80). From birth, normal individuals experience emotions such as fear whenever they are exposed to danger or trauma in daily life. These emotional experiences then induce so-called ‘fear conditioning’ by pairing the stimuli or contexts with danger or trauma. Therefore, emotional responses serve a protective role by producing aversion to contexts associated with danger. Due to their lack of NGF-dependent neurons, patients with CIPA cannot detect various noxious stimuli nor trigger emotional responses to noxious stimuli in the same way as normal individuals. Accordingly, they may be impaired in their ability to modify their behaviors in order to protect their bodies and maintain homeostasis.

Emotion is a strong feeling characterized by various complex reactions with both mental and physical manifestations closely related to activities of the sympathetic nervous system. The ‘fight-or-flight’ response illustrates a strong emotional state associated with an extreme excitation of the sympathetic nervous system. Normal individuals, however, experience emotion to varying degrees in daily life. Most emotional responses occur at levels beneath awareness.

Extreme emotions may have a deleterious effect on health and daily life. Antonio Damasio proposed (81) that emotions are in the loop of reason, and that emotion may assist rather than disturb reasoning processes. Damasio further advanced a hypothesis (known as the ‘Somatic Marker Hypothesis’) that emotions and feelings play critical roles in decision-making via mechanisms he termed ‘somatic markers’ (81); he uses the term ‘emotions’ to denote a collection of changes occurring in both the brain and body that are usually prompted by particular mental contents (81). Feelings are the perceptions of these changes. According to this hypothesis, somatic markers tag neural images involved in reasoning processes with emotions related to past experiences,

thereby conferring differential values upon such images. The human brain uses these somatic and emotional signals to make decisions that conform to previously acquired knowledge.

It is interesting to note that the autonomic nervous system is crucial to achieving the appropriate modification of physiological parameters in the body that generate somatic state that characterizes certain emotions. Indeed, sympathetic skin conduction responses have been used as indices to test the Somatic Marker Hypothesis in the laboratory (81). Damasio also proposed that interoceptive polymodal receptors convey internal milieu and visceral signals from the body's interior to the brain and play critical roles in feelings (82). NGF-dependent neurons mediate reciprocal communication between the brain and the body-proper (Fig. 5). Therefore, these neurons play important roles in the structures and behaviors underlying the Somatic Marker Hypothesis.

Conclusion

Genetic studies have revealed that CIPA results from loss-of-function mutations in the *NTRK1* gene encoding the receptor tyrosine kinase for NGF. This shows that the NGF-TrkA system plays a crucial role in the development and functions of nociceptive reception and the establishment of thermoregulation via sweating in humans. CIPA can also serve as a model to determine the mechanisms of survival and maintenance of NGF-dependent neurons in the autonomic, sensory and central nervous systems and the functions of these neurons in humans. The molecular pathophysiology of CIPA strongly suggests that the NGF-TrkA system is essential for the establishment of peripheral neural networks involved in the interoception and homeostasis that may also underlie certain emotions.

Recent studies indicate that NGF-dependent neurons play crucial roles in brain-immune-endocrine interactions in pain, itch and inflammation and suggest that the NGF-TrkA system is involved in various disease states. Targeting the molecular mechanisms of NGF-TrkA signal transduction is an active area of research that may help to develop novel analgesics and anti-pruritic and anti-inflammatory drugs.

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Figure legends

Fig. 1. Binding of nerve growth factor (NGF) to tropomyosin-related kinase A (TrkA) receptors and intracellular signal transduction.

The binding of the NGF dimer to TrkA stimulates homodimer formation. TrkA is phosphorylated in response to NGF and is essential for intracellular signal transduction. The domain structure of TrkA with 790 or 796 amino acids is shown (23): SP, signal peptide; CC-1 and CC-2, the first and second cysteine clusters, respectively; LRMs, leucine-rich motifs; Ig-1 and Ig-2, the first and second immunoglobulin-like motifs, respectively; TM, transmembrane; JX, juxtamembrane; TK, tyrosine kinase. A single-transmembrane domain divides TrkA into extracellular and intracellular domains. The extracellular domain is important for specific NGF binding. The intracellular domain includes a tyrosine kinase domain. Phosphorylated tyrosine residues-490, 670, 674, 675 and 785 in the TrkA cytoplasmic domain-serve as anchors for binding downstream signaling molecules (25).

TrkA receptors control three major signaling pathways (17). The activation of Ras results in the activation of the mitogen activated protein (MAP) kinase signaling cascade, which promotes neuronal differentiation, including neurite outgrowth. The activation of phosphatidylinositol-3-kinase (PI3-K) through Ras or Gab1 promotes the survival and growth of neurons and other cells. The activation of phospholipase C- γ 1 (PLC- γ 1) results in the activation of Ca^{2+} - and protein kinase C (PKC) -regulated pathways that promote synaptic plasticity. Each of these signaling pathways also regulate gene transcription.

Fig. 2. The location of human *TRKA* mutations associated with congenital insensitivity to pain with anhidrosis.

The human *TRKA* gene, located on chromosome 1 (1q21-q22), is divided into 17 exons and 16 introns (83). The entire sequence is estimated to span at least 23 kb, coding for a protein of 790 or 796 amino acid residues. Six amino acid residues encoded by exon 9 are in the extracellular domain of the neuronal-specific tropomyosin-related kinase A (TrkA) receptor (84). The abbreviations on the bottom row indicate the domain structures (Fig. 1) encoded by the corresponding exon(s) (23, 83). Multiple mutations were detected by our group and are shown here. The numbering system of the mutations

after exon 9 is different from that used previously (37-41). An asterisk denotes the common Japanese founder mutation (G554fs). The three variants in brackets are probably polymorphisms in a particular ethnic background.

Fig. 3. Patients with congenital insensitivity to pain with anhidrosis lack nerve growth factor (NGF)-dependent primary afferent neurons with thin fibers (NGF-dependent primary afferents) and autonomic sympathetic postganglionic neurons.

NGF-dependent primary afferents are dorsal root ganglia (DRG) neurons or trigeminal ganglia (V) neurons with free nerve endings. A subset of neurons in the glossopharyngeal nerve (IX) and the vagus nerve (X) are most likely NGF-dependent neurons. Sympathetic postganglionic neurons innervate blood vessels, piloerector muscles and sweat glands as well as other target organs or tissues in the body. Postganglionic fibers to sweat glands are cholinergic. Triggering factors (shown by bold arrow) may directly or indirectly stimulate NGF-dependent primary afferents. Upon stimulation, these neurons release neuropeptides (SP and CGRP) that modulate inflammation, pain and itch. Sympathetic postganglionic neurons can also influence inflammation.

CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglia; SG, sympathetic ganglion; SP, substance P; STT, spinothalamic tract.

Adapted from Indo (62). This figure is the author's version of the manuscript (62) and is posted in the Kumamoto University Repository System (<http://reposit.lib.kumamoto-u.ac.jp/handle/2298/23889>).

Fig. 4. Nerve growth factor (NGF)-dependent neurons and the brain, immune and endocrine systems.

NGF-dependent primary afferents (P) and sympathetic postganglionic neurons (S) form an interface between the nervous system and the body and play critical roles in mediating cross-talk between the three 'super-systems:' the brain, immune and endocrine systems. NGF-dependent primary afferents report the physiological or pathological status of various tissues in the body to the brain via the spinal cord, and the brain maintains homeostasis in the body via sympathetic neurons and other autonomic, neuroendocrine and behavioral mechanisms. Triggering factors (shown by bold arrow) may directly or indirectly stimulate NGF-dependent primary afferents. Recursive loops

of P from the body indicate signal transduction via the axon reflex. The axon reflex is an efferent function of the NGF-dependent primary afferents in which the release of neuropeptides such as substance P and calcitonin gene-related peptide affects functions in the body, e.g. blood vessels, mast cells (M) and various immune cells. Mast cells reside in various tissues and constitute a critical effector mechanism in inflammation.

The systemic response of the sympathetic nervous system to dangerous signals is known as the 'fight-or-flight' response and activates the stress system in the brain. The peripheral limbs of the stress system are located in the endocrine hypothalamic-pituitary-adrenal (HPA) axis and the efferent sympathetic/adrenomedullary systems (68).

The immune system can affect the brain directly via cytokines in blood flow during inflammatory responses (63, 65, 67, 68). In response to this, the brain affects the immune system via NGF-dependent neurons and the HPA axis. The parasympathetic portion of the autonomic nervous system also plays an important role in the control of immunity and inflammation (not shown). The endocrine system is engaged via the HPA axis in coordinating and controlling complex responses of the brain and the immune system. In accordance with the concept of 'super-systems,' NGF-dependent neurons are considered to form communication routes between the brain, immune and endocrine systems.

Fig. 5. Nerve growth factor (NGF)-dependent neurons mediate reciprocal communication between the brain and the body-proper.

NGF-dependent neurons constitute a part of the neural network for interoception and homeostasis and play important roles in emotions and adaptive behaviors. The diagram is a schematic presentation of the transmission signals that occur between the body-proper and the brain via NGF-dependent neurons, including NGF-dependent primary afferents (P) (interoceptive polymodal receptors) and sympathetic postganglionic neurons (S). The body-proper refers to the organism minus the neural tissues (the central and peripheral components of the nervous system). NGF-dependent primary afferents are DRG neurons or trigeminal ganglia (TG) neurons with free nerve endings. The trigeminal nerve carries sensory information from the face, sinuses, teeth and the anterior portion of the oral cavity. Axons of the trigeminal nerve ganglion cells that process pain and temperature sensations terminate in the spinal nucleus of the trigeminal nerve. A subset of neurons in the glossopharyngeal nerve and the vagus nerve are most likely NGF-dependent neurons (not shown). APG, autonomic preganglionic

neurons; DRG, dorsal root ganglia; SG, sympathetic ganglion; STT, spinothalamic tract.

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