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NGF as a Mediator of Inflammatory Pain

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NGF as a mediator of inflammatory pain

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SUMMARY

The chapter reviews some of recent evidence which suggests that one neurotrophin, nerve growth factor (NGF), is a peripherally produced mediator of some persistent pain states, notably those associated with inflammation. The evidence for this proposal is as follows.

1. The endogenous production of NGF regulates the sensitivity of nociceptive systems. Behavioural and electrophysiological studies have shown that sequestration of constitutively produced NGF leads to decrease nociceptor sensitivity.

2. In a wide variety of experimental inflammatory conditions NGF levels are rapidly increased in the inflamed tissue.

3. The high-affinity NGF receptor, trkA, is selectively expressed by nociceptive sensory neurons particularly those containing sensory neuropeptides such as substance P and CGRP.

4. The systematic or local application of exogenous NGF produces a rapid and prolonged behavioural hyperalgesia in both animals and humans. Exogenous NGF has also been found to activate and sensitize fine calibre sensory neurons.

5. In a number of animal models, much of the hyperalgesia associated with experimental inflammation is blocked by pharmacological 'antagonism' of NGF.

The mechanisms by which NGF up-regulation in inflamed tissues might lead to sensory abnormalities is also discussed. In particular, evidence is reviewd which suggests that increased NGF levels leads to both peripheral sensitization of nociceptors and central sensitization of dorsal horn neurons responding to noxious stimuli.

1. INTRODUCTION

This volume is a testimony to our increasing understanding of the effects and biological roles of neurotrophins. In the adult animal, in particular, our knowledge has grown very rapidly in the last few years. Two main avenues of investigation have been pursued experimentally. First, there is considerable hope that neurotrophins will prove of benefit in the treatment of peripheral neuropathies (as reviewed in McMahon & Priestley 1995). The rationale is that these molecules are known to exert profound survival-promoting effects in the developing animal on all branches of the peripheral nervous system (sensory, motor and autonomic), and they may therefore be of benefit in arresting or reversing peripheral degenerative or atrophic changes associated with disease states. Data from the study of animal models of neuropathy are encouraging, and there are now several clinical trials underway (see McMahon & Priestley 1995).

The second area of intensive study relating to the role of neurotrophins in mature animals has concerned the possibility that one of the neurotrophins, nerve growth factor (NGF), may be an important mediator of some forms of persistent pain. This is the central hypothesis examined in this chapter. Some of the developmental effects of NGF, reviewed elsewhere in this book, are consistent with such a role. For instance, transgenic animals lacking the NGF gene are born with virtually no small calibre primary sensory neurons (most of which are normally nociceptive) and are, as expected, profoundly hypoalgesic (Crowley *et al*. 1994). Transgenic animals lacking the gene for the high-affinity NGF receptor, trkA, appear to exhibit similar deficits (Barbacid 1994), although null mutations of other neurotrophins or neurotrophin receptors do not show these effects (see Barbacid 1994; McMahon & Priestley 1995). Animals which over-express NGF in cutaneous targets from mid-embryonic stages also show changes in pain-related behaviour, but in this case they are hyperalgesic (Davis *et al*. 1993).

A final piece of evidence from developmental studies supporting the idea that the normal development of nociceptor systems depends critically on NGF availability comes from the observation that one class of nociceptor (the A-δ high-threshold mechanoreceptor) fails to develop in rats that are treated with neutralizing antibodies to NGF in the first two postnatal weeks (Ritter *et al*. 1991; Lewin *et al*. 1992*a*, and see Lewin & Mendell 1993).

In this chapter, I will review evidence accumulated in my own laboratory over the last few years, supporting the role of NGF as a mediator of inflammatory pain in the adult rat. I will present supporting (or conflicting) evidence from the literature where appropriate, but I make no attempt to review systematically all relevant literature. The chapter by Professor Woolf reviews other related experiments in this field. The work from both our laboratories converges on broadly similar conclusions.

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2. EVIDENCE THAT NGF ACTS AS MEDIATOR OF PERSISTENT PAIN STATES IN ADULT MAMMALS

There are now multiple and independent lines of experimental work suggesting that NGF critically interacts with pain-signalling systems. Some of the evidence is correlative, relating to levels of endogenously produced NGF, and the distribution of its high affinity receptor, trkA, and some relates to the effects of exogenously administered NGF. Some of the evidence is circumstantial, or simply consistent with the proposed role of NGF, while some is direct. The main lines of evidence are summarised in the sections below:

(*i*) *NGF maintains nociceptor sensitivity* in vivo

NGF is constitutively expressed in the adult animal in many peripheral tissues of the body. The levels expressed are generally very low (Shelton & Reichardt 1984). There are some exceptions, such as the male mouse salivary gland, but the function of the high levels of NGF in this tissue remains obscure. A variety of cell types produce NGF. The main supply in normal skin appears to originate from keratinocytes (Albers *et al.* 1994). In the deep tissues of the body, smooth muscle cells can manufacture the protein (Steers *et al.* 1991). Although normal levels are low in adult tissue, they are sufficient to exert strong biological effects on the peripheral innervation of the body. One piece of evidence is that peripherally produced NGF protein is retrogradely transported by peripheral nerves (Otten 1991; DiStefano *et al.* 1992; Donnerer *et al.* 1992). Other evidence comes from the use of autoimmunisation experiments (Otten 1979; Gorin & Johnson 1980; Rich *et al.* 1984; Gorin *et al.* 1990), which suggest that the levels of neurotransmitters in sympathetic neurons depend upon constitutively produced NGF. These experiments also suggest that in the adult, unlike the developing animal, NGF is not necessary for the survival of sympathetic and sensory neurons.

We have recently examined the normal biological role of NGF using a synthetic fusion protein, a trkA-IgG, capable of sequestering NGF (McMahon *et al.* 1995*a*). The molecule, consisting of two extracellular domains of trkA dimerized via the association of their respective Fc regions, was constructed recombinantly (Shelton *et al.* 1995). The molecule was capable of selectively 'antagonising' the survival-promoting effects of NGF (but not NT3 or BDNF) in an *in vitro* survival assay of embryonic sensory neurons (McMahon *et al.* 1995*a*). We have infused this anti-NGF 'receptorbody' subcutaneously into the hindpaw of adult rats over a period of weeks, using mini-osmotic pumps. The animals were tested on a regular basis for their sensitivity to noxious thermal stimuli. After treatment for 4–5 days, the animals started to show prolonged latencies of response to the noxious heating. That is, they became hypoalgesic. After two weeks of continuous treatment, the animals also showed greatly reduced responsiveness to a chemical irritant, capsaicin. Together, these results suggest that normal levels of NGF are important regulators of the sensitivity of pain-signalling systems. The mechanism by which this occurs is not clear. One possibility is that NGF deprivation leads to death of primary sensory neurons. However, this would not explain the fact that the behavioural hypoalgesia associated with trkA-IgG treatment showed a complete recovery some days after treatment finished. Previous auto-immunisation data, quoted above, also speak against it. Another possibility is that the production of the appropriate transducers-receptors by the sensory neurons depends upon the supply of NGF. In support, there is good evidence that the capsaicin sensitivity of cultured sensory neurons is regulated by the levels of NGF in the medium (Winter *et al.* 1988; Bevan & Winter 1995). A third possibility is that NGF may regulate the morphology of terminal arbors of sensory neurons in skin, and in the absence of available NGF these arbors may retract. The work of Diamond and colleagues suggests that in other contexts the levels of available NGF can do just this (Diamond *et al.* 1987, 1992*a*, *b*), and we now have preliminary evidence to support this claim (Bennett *et al.* 1995). Whatever the mechanism of the effect, the implication is that the availability of NGF has the capacity to regulate strongly the responsiveness of nociceptive systems.

(*ii*) *NGF up-regulated in a variety of inflammatory conditions*

A key component of the hypothesis that NGF is a mediator of inflammatory pain is that the levels of the protein will be increased in appropriate tissues at appropriate times in inflammatory states. In fact, there is now considerable and compelling evidence that just such an increase in NGF expression is a ubiquitous response in inflammation. In a variety of animal models of inflammation, including those produced by Freund's adjuvant (Donnerer *et al.* 1992; Safieh-Garabedian *et al.* 1995), subcutaneous carrageenin (Westkamp & Otten 1987; Otten 1991; Aloe *et al.* 1992), and in a rat model of cystitis (Andreev *et al.* 1993; Oddiah *et al.* 1995, and see below), NGF expression is increased. NGF also appears to be up-regulated in the joints of human arthritic patients (Aloe *et al.* 1992). The increased NGF expression has been seen by a number of different techniques including: an increase in NGF protein in the inflamed tissue; an increase in the amount of NGF protein in nerves supplying the affected tissue; an increase in mRNA levels measured by in situ hybridisation and by reverse transcriptase polymerase chain reaction RT-PCR. The extra NGF produced in inflamed tissue may derive from a variety of sources. Immune cells (lymphocytes, macrophages, mast cells) appear a rich potential source of NGF (Brown *et al.* 1991; Aloe *et al.* 1992; Leon *et al.* 1994; Santambrogio *et al.* 1994). However, other cell types such as fibroblasts or Schwann cells in nerve in the inflamed tissue are other possible sources (Heumann *et al.* 1987; Matsuoko *et al.* 1991) .

We have looked in some detail at NGF expression in one particular model: a rat model of cystitis. Here, an

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acute sterile inflammation is precipitated by the brief intravesical administration of turpentine oil in anaesthetized female rats (McMahon & Abel 1986; McMahon *et al.* 1995*b*). Starting within an hour, plasma extravasation and oedema are seen, and leucocytes migrate into the tissue. The treated animals show features typical of cystitis is humans: increased bladder motility and frequency of micturition, and signs of abdominal hyperalgesia and ongoing discomfort. These behavioural and reflex changes begin within about an hour and persist for up to 24 hours. Electrophysiological experiments also show sensitization of primary afferent and spinal cord neurons innervating the bladder, with a similarly rapid onset (Habler *et al.* 1988, 1993; McMahon 1988; see also McMahon *et al.* 1995*b*). In the same model, *in situ* hybridisation with a riboprobe specific for NGF mRNA showed a very marked increase starting within two hours of the inflammatory stimulus, peaking shortly thereafter and returning to baseline levels within 24 hours (Andreev *et al.* 1993). More recently, we have also seen equivalent increases with RT-PCR and a sensitive ELISA for NGF protein (Oddiah *et al.* 1995). Thus, NGF up-regulation closely parallels the sensory and reflex abnormalities in this model.

(*iii*) *trkA receptors are found selectively on nociceptive afferents*

Because the biological effects of NGF are believed to be mediated largely or exclusively through the high-affinity trkA receptor, the expression of this receptor is likely to indicate those neuronal populations which might be directly affected by the neurotrophin. There is now a compelling body of evidence to suggest that the trkA receptor is expressed in adult animals selectively by small calibre, largely nociceptive afferents.

The expression of trkA receptors on sensory neurons has been studied using a number of techniques, including *in situ* hybridisation for trkA mRNA (Carroll *et al.* 1992; Verge *et al.* 1992; Mu *et al.* 1993; McMahon *et al.* 1994; Wright & Snider 1995), high-affinity binding of labelled NGF (Richardson *et al.* 1986; Verge *et al.* 1989*a*, *b*, 1990, 1992), retrograde transport from peripheral nerve to dorsal root ganglia of iodinated NGF (Richardson & Riopelle 1984; DiStefano *et al.* 1992) and immunohistochemistry (Averill *et al.* 1995). These studies all agree that in the L4 and L5 dorsal root ganglia, about 45% of adult neurons express trkA. These cells are mostly of small diameter.

In a recent study we examined the coexpression of trkA with various neurochemical markers (Averill *et al.* 1995). DRG cells can be divided into three minimally overlapping subgroups. First, the population traditionally described as 'large light' can be identified by the anti-neurofilament antibody RT97 (Lawson *et al.* 1984). These cells, about 40% of the total, have mostly myelinated axons, and in the periphery are presumably connected to mechanosensitive endings such as Pacinian corpuscles, hair follicle afferents and muscle spindles (for reviews see Lawson 1992; Willis & Coggeshall 1991). A second population of DRG cells contain cell surface glycoconjugates with terminal D-galactose residues and can be identified with markers such as the monoclonal antibody LA4 (Alvarez *et al.* 1989) and the lectin *Griffonia simplicifolia* IB4 (Silverman & Kruger 1990). These neurons, about 30% of the total (Alvarez *et al.* 1991), have unmyelinated axons, do not show RT97 immunoreactivity, and are likely to innervate predominantly nociceptors and thermoreceptors (Willis & Coggeshall 1991). The third population of DRG cells consists of those that constitutively synthesize neuropeptides. The best marker for this group is the neuropeptide that is expressed by the largest number of DRG cells, namely CGRP. About 45% of lumbar DRG cells contain CGRP immunoreactivity (Lawson 1992) and peptides such as substance P, somatostatin and galanin coexist with CGRP. CGRP immunoreactive cells are predominantly small with unmyelinated axons and hence form the other half of the 'small dark' population, again likely to innervate predominantly nociceptors and thermoreceptors.

A striking feature is that trkA expression corresponds almost perfectly with the CGRP population. trkA immunoreactive cells (92%) were found to contain CGRP (Averill *et al.* 1995). In contrast the non-peptide LA4/IB4 population was largely trkA negative with only 6% of LA4 immunoreactive cells showing trkA immunoreactivity. The overlap observed between trkA and the markers RT97, IB4 and LA4 corresponds closely to the known overlap between CGRP and these markers.

The expression of high-affinity NGF receptors appears to vary between functionally distinct populations of sensory neurons. Using *in situ* hybridisation for trk mRNAs, we recently observed that relatively few afferent neurons innervating skeletal muscle expressed trkA, whereas those innervating a visceral target, the urinary bladder, were nearly all trkA expressing (McMahon *et al.* 1994). Interestingly, this latter population also appeared to co-express trkB, the high-affinity receptor for two other members of the neurotrophin family, BDNF and NT4/5. In all cases, however, trkA was found predominantly in small neurons which are known to be responsive to nociceptive stimuli.

Together these results strongly suggest that altered levels of NGF have the capacity to directly interact with specifically pain-signalling peripheral sensory systems.

(*iv*) *Exogenous NGF produces hyperalgesia*

There is direct evidence that exogenous NGF can alter pain-related behaviour. Lewin *et al.* (1993, 1994) studied the effects of a single systemic dose of 1 mg/kg of NGF on the sensitivity of the animals to noxious thermal and mechanical stimuli. They found that this single dose elicited two phases of hyperalgesia. The first appeared less than 30 minutes after NGF treatment, while the second took several hours to emerge and persisted for several days. Thermal hyperalgesia was present in both phases but mechanical hyperalgesia

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was found only during the delayed, second, phase. Lewin *et al*. suggested that the first phase arose because of a peripheral action of NGF, whereas the second required changes in the central processing of nociceptive information. These conclusions were based partly on arguments relating to the timing of the effects and partly on the effects of pharmacological manipulations (see Lewin 1995).

We have recently undertaken related studies in which small doses of NGF were injected subcutaneously into the hindpaws of adult rats (Andreev *et al*. 1995). Doses of in the range of 50–500 ng produced a dose dependent thermal hyperalgesia, which appeared within 30 minutes of treatment and lasted for a number of hours. The effect was large in magnitude and observed in experiments where the experimenter was blinded to the treatment. NGF was probably acting at the site of injection and not systemically in these experiments since the hyperalgesia was seen only ipsilaterally.

Injections of NGF to human volunteers also leads rapid onset and marked increased sensitivity to painful stimuli (Petty *et al*. 1994). Intravenous injections (at very low doses of 1 μg/kg) produced a widespread aching pain in deep tissues. Subcutaneous injections also produced hyperlagesia at the site of injection. These effects developed quickly (within 60 minutes in some cases) and persisted for hours or a few days.

The hyperlagesia produced by NGF can be sustained. Transgenic animals which continuously overexpress NGF in skin from mid-developmental stages are hyperalgesic when tested as adults (Davis *et al*. 1993). We have examined the effects of chronic (two-week) subcutaneous infusions of NGF into adult animals (Al Sahili *et al*. 1995), or repeated daily subcutaneous injections. In both cases the thermal hyperalgesia associated with these low dose treatments was maintained throughout the treatment period.

(*v*) *Exogenous NGF activates and sensitises nociceptive systems*

One would expect that the ability of NGF to induce behaviourial hyperalgesia, described above, would be reflected in physiological changes in nociceptive systems. And indeed there are several strands of supporting experimental evidence. One observation is that local administration of NGF leads to a restricted neurogenic extravasation of plasma proteins into the tissue. We have monitored this extravasation using the Evan's blue technique, following NGF treatment of skin and bladder (Andreev *et al*. 1995; Dmitrieva & McMahon 1996). In both cases a modest extravasation resulted within tens of minutes of NGF application. Because this extravasation is absent in animals treated neonatally with capsaicin (and therefore lacking most unmyelinated afferent fibres), the extravasation is likely to be neurogenic in origin. That is, NGF is normally capable of inducing a response analogous to a component of the triple response of Lewis. It is known that neurogenic extravasation depends upon neuropeptides released from the peripheral terminals of nociceptors following their activation. Thus, NGF appears capable of acutely activating some primary sensory nociceptors.

We also have direct electrophysiological evidence of peripheral activation and sensitization of thin calibre afferent fibres by NGF. In anaesthetized rats we characterised the response properties of thin (Aδ and C) fibres innervating the urinary bladder (Dmitrieva & McMahon 1996). We then exposed the peripheral terminals of these afferents to NGF by injecting it intravesically. We found that within 30 minutes of this exposure, most afferent neurons were sensitized to bladder distension. This was true for both Aδ and C fibres, and included some fibres that initially had no mechanosensitivity (i.e. were of the type known as 'silent' nociceptors: McMahon & Koltzenburg 1994). The sensitization persisted for the duration of recordings (up to three hours). Many of the fibres also developed low levels of ongoing activity. These changes were very similar to those reported following chemical inflammation of the urinary bladder, as described in §2 (*ii*), above. That is, the time course and nature of changes in bladder primary afferent neurons in a model of cystitis are consistent with their mediation by NGF.

There are other data that suggest that NGF can activate and sensitise peripheral nociceptive fibres. Firstly, the application of NGF into the urinary bladder, as described above, leads to induction of the proto-oncogene c-fos in dorsal horn neurons (N. Dmitrieva & S. McMahon, unpublished data). The number of cells activated and their lamina distribution was again very similar to that seen after chemical inflammation of the urinary bladder.

We have also examined electrophysiological changes after NGF treatment of somatic tissues (Andreev *et al*. 1994). In anaesthetized rats we recorded from dorsal horn neurons with receptive fields on the hindpaw and activated by noxious heating. Small doses of NGF (500 ng) were then injected subcutaneously into the centre of the receptive fields. Starting within 20 minutes most neurons showed a progressive increase in ongoing activity and responsiveness to noxious heating. The most parsimonious explanation for these findings is that the NGF caused a peripheral sensitization of nociceptors which was then reflected in enhanced responses of dorsal horn neurons.

Recently, Rueff *et al*. (1995) have studied the responsiveness of cutaneous nociceptors using an *in vitro* preparation and have found that topical administration of NGF produces a heat sensitization in a about 20% of afferent nociceptors.

These results, utilising different techniques, all suggest that increased levels of NGF are capable of rapidly sensitizing peripheral terminals of nociceptors.

(*vi*) *Sequestration of NGF blocks inflammation-induced hyperalgesia*

The evidence presented above is consistent with the suggestion that NGF is a mediator of inflammatory pain states. However, to demonstrate the biological role of endogenous NGF, one requires pharmacological 'antagonism'. We currently have no selective antag-

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onists of the trkA receptor. Several previous studies have used neutralising antibodies or auto-immunisation procedures to study the effect of endogenous NGF (see §2 (*i*), above). For the most part these been directed at questions of the effects of NGF on the regulation of phenotype, survival and collateral sprouting of sympathetic neurons and primary afferents. Because our knowledge of the neurotrophin family and their receptors is so new, the relatively early immunisation studies were hampered by unknown, and unknowable, cross-reactivity with other neurotrophins. Some antibodies that have been subsequently tested have shown such cross-reactivity (Murphy *et al.* 1993). The use of polyclonal antibodies is further limited by the supply available. Auto-immunisation procedures produce titres of antibody that vary with time and from animal to animal and cannot be used to demonstrate threshold or saturating levels. For these reasons we have used, in *in vivo* experiments, a synthetic trkA-IgG fusion molecule capable of sequestering and neutralising NGF but not other neurotrophins (see §2 (*i*) above).

We have asked if this molecule can block the sensory abnormalities that develop in two models of inflammation: that produced by subcutaneous carrageenin and the rat model of cystitis described previously (see §2 (*ii*) and §2 (*v*), above).

In the model of cystitis we have measured the progressive increase in bladder reflex excitability that occurs with inflammation. Normally, slow filling of the bladder results initially in gradual increases in intravesical pressure, but at some critical point a series of large, active, CNS-generated, micturition contractions begin. These micturition contractions are initiated by activity in afferent neurons innervating the urinary bladder, and so indirectly reflect the sensitivity of sensory systems (McMahon 1986). At the onset of experimental inflammation, the excitability of bladder reflexes measured in this way rises, and micturition contractions are initiated at lower distending volumes. When animals are pre-treated systemically with the NGF sequestering molecule (1 mg/kg) the hyperreflexia associated with inflammation fails to develop. Pretreatment with a trkB-IgG, which is a similar molecule but without NGF-sequestering capacity, does not have this effect. The trk-IgGs do not appear to cross the blood brain barrier. Thus, these data suggest that in this model of inflammation the sensory changes occurring with inflammation are critically dependent on peripheral up-regulation of NGF.

In the carrageenin model of inflammation, subcutaneous injections of this agent into a hindpaw produces a marked and persistent thermal hyperalgesia of the treated paw. When we undertook identical experiments except that trkA-IgG was mixed with the inflammatory agent, most of the expected hyperalgesia did not develop (McMahon *et al.* 1995*a*). The block of carrageenin hyperlagesia by trkA-IgG was dose dependent and not seen with control IgG molecules. The conclusion from these experiments is again that these sensory abnormalities developing with inflammation is critically dependent upon the production of NGF in the inflamed tissue.

Similar conclusions have been reached in two other recent studies that have used neutralizing antibodies to NGF (Lewin *et al.* 1994; Woolf *et al.* 1994; see chapter by Woolf). Both report that hyperalgesia associated with experimental inflammation is blocked when NGF is sequestered. These experiments provide the most compelling reasons for believing that up-regulation of NGF in inflammatory states is of key functional importance for the abnormal pain sensations that arise. The way(s) in which this might come about are discussed in the next section of this review.

3. MECHANISMS OF NGF ACTION IN INFLAMMATORY PAIN

The evidence presented above can be interpreted in a number of ways. However, at present there are two ways in which the data fits most convincingly into our framework of understanding of mechanisms of persistent pain. These are described below and illustrated schematically in figure 1.

(*i*) *The peripheral sensitization of nociceptors*

There is now a large body of evidence (reviewed in Koltzenburg 1995; Reeh & Kress 1995) that the encoding properties of primary sensory nociceptors are modifiable. Most notably, a number of pathophysiological states, particularly those caused by tissue injury or inflammation, are associated with the tonic activation and sensitization of these sensory neurons, in which they lower their thresholds for activation and respond more vigorously than normal to noxious stimuli. Clearly, this increased sensitivity of nociceptors is likely to contribute to the ongoing pain and hyperlagesia seen in these pathophysiological states. It is also now clear that a wide variety of chemicals, including prostaglandins, bradykinin, serotonin, histamine and even hydrogen ions can mimic this sensitization of nociceptors. Many of these agents are released into damaged tissue.

It is therefore not a radical suggestion that NGF itself might also lead to this sensitization. The rapid onset of hyperalgesia after subcutaneous injections of NGF strongly suggests a peripheral action. The ability of NGF to induce neurogenic extravasation (Andreev *et al.* 1995; Dmitrieva & McMahon 1996) also strongly suggests such a role. Finally, of course, the direct electrophysiological observations of sensitization of primary sensory neurons clearly demonstrates this effect (Dmitrieva & McMahon 1996; Rueff *et al.* 1995). Given that many nociceptors express the trkA receptor, it is possible that sensitization occurs following the direct binding and activation of this receptor by NGF.

However, there are other cellular elements in peripheral tissues which express the trkA receptor and it is therefore possible that sensitization of nociceptors arises indirectly. TrkA receptors are known to be expressed by both sympathetic postganglionic neurons and by some mast cells (Horigome *et al.* 1993; Thoenen 1991), and indeed NGF is known to be a potent

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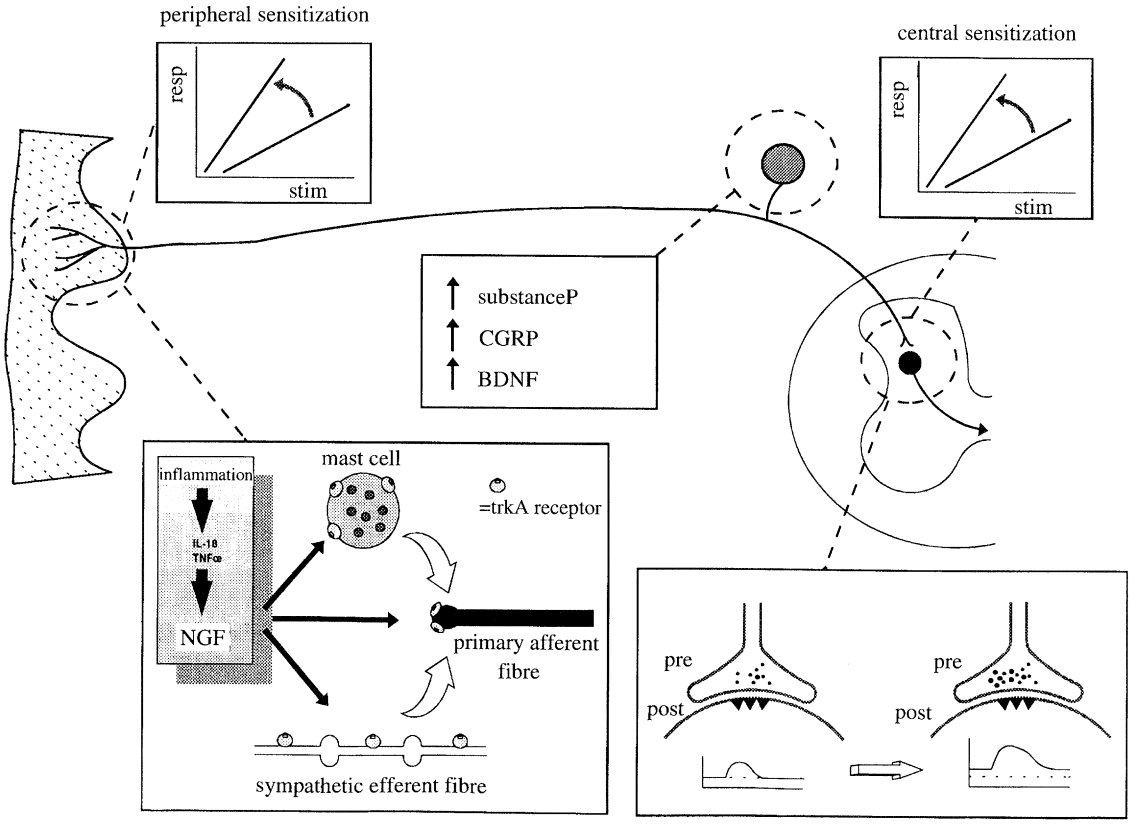

Figure 1. Schematic illustration of the mechanisms by which NGF may affect pain-signalling systems. See text for details.

degranulator of mast cells (Horigome *et al.* 1993). There is good evidence that mast cell products (such as histamine and serotonin) are capable of sensitizing nociceptors (see Reeh & Kress 1995). There is also a body of evidence that, in other contexts, the chemical activation of sympathetic postganglionic fibres can lead to the release of a number of products of arachadonic acid metabolism, that are capable of producing hyperalgesia (reviewed in Heller *et al.* 1994; Koltzenburg & McMahon 1991). There is now experimental evidence suggesting that at least a large component of the peripheral sensitizing effect of NGF is indirect. Thus the rapid onset hyperlagesia produced by NGF is largely blocked in sypathectomized animals (Andreev *et al.* 1995) or in animals pre-treated with the mast cell degranualtor 48/80 (Lewin *et al.* 1994). The cascade of events leading to peripheral sensitization by NGF is illustrated schematically in figure 1.

(*ii*) *The central sensitization of dorsal horn neurons*

Notwithstanding the evidence presented in the preceding section, there are reasons to believe that NGF may have an important impact on pain signalling systems other than by inducing peripheral sensitization. One reason is that NGF is retrogradely transported by trkA expressing neurons and is known to exert major effects on gene expression in those cells (see below). A second is that the later components of hyperalgesia induced by systemic injections of NGF not readily explained by peripheral mechanisms and appear to have a central component (Lewin *et al.* 1994; Lewin 1995). A main development in the field of pain research over the last decade or so is the recognition that the central relay of nociceptive information in the spinal cord is itself rather plastic. In particular, there are now many examples of increases in excitability of these central neurons triggered by peripheral injury. These central changes, dubbed central sensitization, in most instances appear to share common physiology and pharmacology (see McMahon *et al.* 1993). There is now some circumstantial and direct to suggest that peripheral increases in NGF levels can also activate this process.

The evidence for this mechanism, illustrated in figure 1, comes in several parts. First, NGF modulates levels of number of neurotransmitters-neuromodulators in sensory neurons. *In vivo* (Kuraishi *et al.* 1989; Donnerer *et al.* 1992; Leslie *et al.* 1995) and *in vitro* (see Lindsay 1992), substance P and CGRP levels are increased by NGF. The increased production of these peptides is reflected by their increased levels in the central terminals of nociceptors. It is known that many forms of central sensitization appear to be depend upon the sensory neuropeptides substance P and CGRP released from the central terminals of primary afferent nociceptors with activity (see McMahon *et al.* 1993). One can therefore hypothesise that the increased levels of these peptides by NGF is a key intermediate step in the generation of central sensitization.

Increased retrograde transport of NGF also has other important effects on small diameter sensory neurons. These include the apparent increased expression of receptors expressed by the neurons (e.g.

capsaicin and GABA, Bevan & Winter 1995). Altered receptor expression on both peripheral and central terminations of nociceptors is likely to have important functional consequences for information processing. The expression of another neurotrophin, BDNF, is also known to be regulated by NGF (Apfel *et al.* 1994; G. Michael, J. Priestley & S. McMahon, unpublished observations). The consequences of this are only speculative at present, but there possibilities of autocrine or (Acheson *et al.* 1995) paracrine (Acheson *et al.* 1995 and see this volume) effects and even of central release followed by post-synaptic actions (i.e. acting as a neurotransmitter).

We also have direct electrophysiological evidence of central sensitization triggered by NGF. In one study (Lewin *et al.* 1992*b*) we delivered NGF to a peripheral target for two weeks by mini-osmotic pumps and then evaluated the excitability of spinal cord neurons to activation of the treated afferents. We found a significant increase in central excitabilty to inputs from both unmyelinated afferents (which are likely to retrogradely transport NGF) and also to activation of myelinated afferents, themselves likely not to be sensitive to NGF. A recent report by Thompson *et al.* (1995) used an *in vitro* preparation to assess the consequences of prior systemic NGF exposure. They too observed a generalised increase in spinal neurone excitability. They also found that the pharmacology of the NGF-induced central sensitization was the same as other forms of central sensitization in that it was blocked by antagonists of both the NMDA receptor the substance P receptor.

The dual mechanisms of peripheral and central sensitization are, of course, not mutually exclusive, but they may cooperate in the genesis of abnormal pain sensibility in cases of increased production of NGF. One would expect the time course of effect to differ, however, given the extra transport time required for effects mediated by altered gene expression.

4. CONCLUSIONS

In this chapter I have presented some of the recent lines of experimental evidence suggesting a role for NGF as a mediator of some persistent pain states. However, there are still several unresolved issues. First, most of the experimental studies have, often of necessity, used models of relatively short-lasting inflammation, typically measured only in days. In contrast, many forms of clinically relevant persistent pain have very much longer durations. We do not know if the effects of NGF, described in this chapter, persist over such long time courses, or conversely whether other actions of NGF may arise with time, for instance the anatomical remodelling of peripheral or even central terminals of nociceptive sensory neurons.

It is also currently somewhat unclear where NGF production fits in the cascade of chemical change in inflamed tissue. In terms of 'up-stream' events, there is now evidence that two cytokines, TNFα and IL-1β, are necessary intermediates leading to the production of NGF in inflammation. It is well recognized that both these cytokines are themselves released into inflamed tissues. We have now found (unpublished observations) that small doses of TNFα injected subcutaneously produce marked thermal and mechanical hyperlagesia that is blocked by sequestering NGF, and that antibodies to TNFα block the hyperlagesia seen in carrageenin inflammation. A similar repertoire of effects has been reported for IL-1β (Safieh-Garabedian *et al.* 1995). Whether these two cytokines act serially or in parallel is not known.

There is also some uncertainty about the events 'downstream' to NGF production. The peripheral effects of NGF may be largely indirect. There is clear evidence that a variety of mediators, including prostaglandins and other products of arachidonic acid metabolism, bradykinin, histamine, and serotonin may contribute to inflammatory pain. Given the evidence for the involvement of both mast cells and post-ganglionic sympathetic terminals in NGF actions, it is feasible that NGF up-regulation is simply 'upstream' to the production of these classical mediators. The failure of existing analgesics to control adequately many forms of inflammatory pain may well stem from their targeting individual 'downstream' mediators. Therefore, strategies aimed at controlling the means of production or action of NGF may represent an important new strategy in the treatment of inflammatory pain states.

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