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Phenotypic Modification of Primary Sensory Neurons: The Role of Nerve Growth Factor in the Production of Persistent Pain

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Phenotypic modification of primary sensory neurons: the role of nerve growth factor in the production of persistent pain

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SUMMARY

Inflammation results in an early and maintained elevation in nerve growth factor (NGF) levels in inflamed tissues. Neutralizing the action of the increased NGF with specific anti-NGF antibodies substantially diminishes inflammatory hypersensitivity, indicating that this neurotrophin is a key mediator in the production of inflammatory pain. The hyperalgesic actions of NGF may in part be the consequence of an increase in sensitivity of the peripheral terminals of high threshold nociceptors either as a result of a direct action of NGF on trkA expressing sensory fibres or indirectly via the release of sensitizing mediators from trkA expressing inflammatory cells and postganglionic sympathetic neurons. NGF is also, however, retrogradely transported in sensory neurons to the dorsal root ganglion where it alters transcription of a number of proteins and peptides. This chapter reviews evidence suggesting that an NGF-mediated modification of gene expression in the dorsal root ganglion during inflammation is central to the pathophysiology of persistent pain. The phenotype changes produced by NGF during inflammation include elevation of neuropeptides which may amplify sensory input signals in the spinal cord and augment neurogenic inflammation in the periphery and the upregulation of growth related molecules which may lead to a hyperinnervation of injured tissue by promoting terminal sprouting.

1. INTRODUCTION

Primary sensory neurons represent the interface between the external environment and the central nervous system (CNS). To detect and transfer particular features of the stimuli that impinge upon the body to the CNS they have highly specialized adaptations that include the heterogeneous expression of a broad number of ion channels, receptors, neurotransmitters and neuromodulators as well as the establishment of highly ordered patterns of innervation of the peripheral target and central neurons. Considerable effort has been directed towards studying those intrinsic and extrinsic factors that enable a neural crest progenitor cell to differentiate into a mature, specialized sensory neuron. Clearly neurotrophins have a major role, both in terms of differentiation and survival, which manifests during the establishment of neuron-target interaction in development. What has been less prominently considered is the possibility that the phenotype of the mature differentiated neuron in the adult is not fixed and that phenotypic modification represents an important element in the alteration of sensory neuron function in different pathological circumstances.

The first indication of phenotypic modification in adult primary sensory neurons comes from studies investigating the effect of disrupting contact of the neuron with its target by cutting the peripheral axon (reviewed in Hokfelt *et al.* (1994). Peripheral axotomy results in a characteristic set of changes that include the down-regulation of a number of neuropeptides including substance P and calcitonin gene related peptide (CGRP) and the novel expression of other neuropeptides like vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY) and galanin. As many, but not all, of these chemical changes can be prevented by administration of neurotrophins (Verge *et al.* 1995), this has been interpreted as demonstrating a role for target-produced growth factors in maintaining the normal phenotype of sensory neurons.

What of the reverse situation, when sensory neurons are exposed to increased levels of neurotrophins in their target tissue. Does this change the phenotype of the neurons and is this of any significance for their normal functional operation?

The purpose of this paper is to review data indicating that peripheral inflammation is accompanied by a substantial increase in NGF production and that NGF is a key mediator in the production of inflammatory hypersensitivity. I will present data in favour of an NGF-mediated alteration in chemical phenotype of primary sensory neurons as representing an essential component of the sensory alterations that occur during inflammation and that manifests as inflammatory pain or hypersensitivity.

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2. NGF AND HYPERALGESIA

That NGF may have the capacity in the adult to increase sensitivity to noxious stimuli, that is produce hyperalgesia, first emerged from the studies of Mendell and colleagues, where they showed that large doses of systemically administered NGF produced both thermal and mechanical hyperalgesia in the rat (Lewin *et al.* 1993). Similar findings have been reported in mice (Della Seta *et al.* 1994) and humans (Petty *et al.* 1994). Local injections also produce sensitivity changes in rats (Woolf *et al.* 1994) and humans (Petty *et al.* 1994). Intraplantar injections of 2–2000 ng NGF, result in a relatively short-lived increased in mechanical and thermal hypersensitivity (figure 1*a*) which, except at the highest dose, is over within 24 h. The onset of the hypersensitivity changes is too early for a transcription-related change as the NGF would need to be retrogradely transported from the site of injection to the cell bodies of the sensory neurons in the lumbar dorsal root ganglia, which at the maximal fast axonal transport rate, would take ~ 5–6 h. After transcription any novel protein would then have to be transported to the peripheral or central terminal before it could effect a change in function. It is likely, therefore, that the immediate sensitivity changes produced by NGF are peripheral (see later).

Although the sensitivity changes produced by a 200 ng intraplantar injection of NGF are transient, it does produce alterations in the phenotype of sensory neurons innervating the site of the NGF administration. The levels of the neuropeptides substance P and CGRP are both elevated in the sciatic nerve post NGF injection (Donnerer *et al.* 1992; Woolf *et al.* 1994) and there is a very substantial increase in the number of dorsal root ganglion cells which stain positive for preprotachykinin A and CGRP mRNA (Leslie *et al.* 1995) (figure 1*b*). This data confirms the suggestion

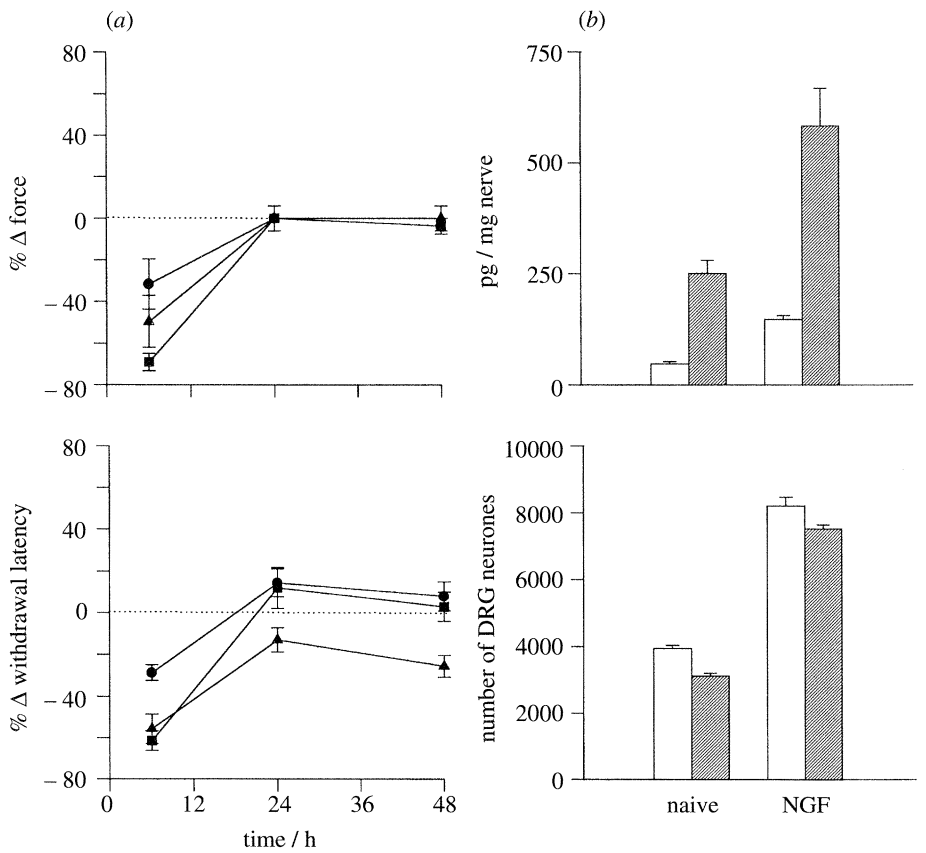

Figure 1. (*a*) Effects of intraplantar NGF (200 ng) on thermal and mechanical sensitivity in the rat. Mechanical sensitivity (top panel) was measured by the change in threshold for eliciting a flexion withdrawal reflex using von Frey hairs. Thermal sensitivity (bottom panel) was measured by the change in reaction time in a hot-plate test (50 °C). Data have been normalised to the pre-injection levels (means ± s.e.m., $n = 5$). Circles represent 2 ng; squares represent 200 ng; triangles represent 2 μg. (*b*) Effect of intraplantar NGF on the expression of substance P(SP) and calcitonin gene related peptide (CGRP) (top panel) in the sciatic nerve innervating the injected paw (measured by ELISA) and by changes in the number of L4 dorsal root ganglion (DRG) cells expressing preprotachykinin A or CGRP (bottom panel) (measured by non-isotopic *in situ* hybridization). Modified from Woolf *et al.* (1994) and Leslie *et al.* (1995). Open area: SP; hatched area: CGRP.

made earlier from culture work that the level of substance P in adult dorsal root ganglion cells is controlled by the level of NGF in the target (Lindsay *et al.* 1989), although cultured cells are necessarily axotomized and might not react in the same way as intact neurons *in vivo*.

3. INFLAMMATION AND NGF

Cell culture experiments have shown that a number of cell types can express NGF (Matsuoka *et al.* 1991; Hattori *et al.* 1993; Leon *et al.* 1994) and that the inflammatory cytokine interleukin 1-β and other growth factors, particularly TNFα are potent inducers of NGF production (Hattori *et al.* 1993). This is also true *in vivo*, where Il-1β administration induces local NGF production (Safieh-Garabedian *et al.* 1995). It is not surprising, given that inflammation results in a massive increase in IL-1β levels (Safieh-Garabedian *et al.* 1995) that it results in an elevation of NGF in the inflamed tissue. Elevated NGF has been described in the distal stumps of degenerated nerves (Heumann *et al.* 1987), in inflammatory exudates (Weskamp & Otten 1987) in the synovium of inflamed joints (Aloe *et al.* 1992), in skin wounds (Constantinou *et al.* 1994) and after adjuvant inflammation (Woolf *et al.* 1994). Figure 2 illustrates the changes in NGF protein present in the skin and in the sciatic nerve after adjuvant inflammation. The latter indicates that the increased local production of NGF results in retrograde transport of the neurotrophin (DiStefano *et al.* 1992), presumably in trkA expressing sensory fibres (McMahon *et al.* 1994).

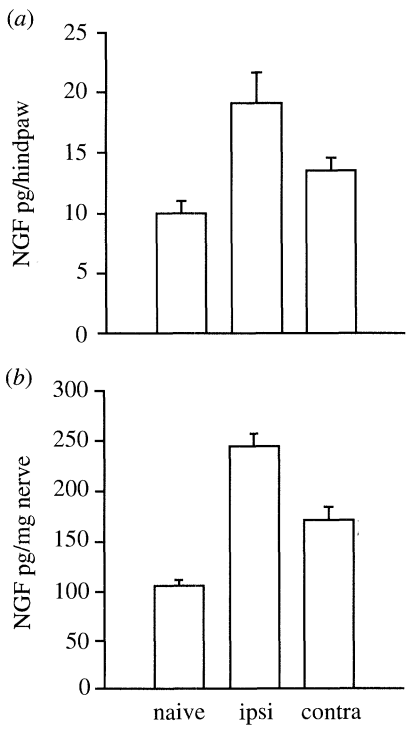

Figure 2. Inflammation produced by a local injection of complete Freund's adjuvant into one hindpaw of a rat results at 48 h in a significant increase in NGF levels, measured by ELISA, in: (*a*) the inflamed skin; and (*b*) the sciatic nerve innervating the site of the inflammation. Modified from Woolf *et al.* (1994).

Does the increase in NGF lead to contribute to inflammatory hypersensitivity? The answer is an unequivocal yes. The administration of a neutralizing anti-NGF serum prior to the induction of adjuvant inflammation significantly prevents the establishment of mechanical and thermal hypersensitivity (Woolf *et al.* 1994), as illustrated in figure 3*a*. This finding has been substantiated by Mendell's group (Lewin *et al.* 1994) and by McMahon and colleagues using a novel approach of a trkA-IgG fusion protein to compete for the NGF.

It is conceivable that NGF may only be an early mediator in the production of inflammation hypersensitivity. To test this, anti-NGF serum was administered after the induction of inflammation at a time when the hypersensitivity was fully developed. Figure 3*b* illustrates how in this case, thermal hypersensitivity is substantially and significantly reduced an hour after the anti-NGF serum. This implies that an ongoing supply of NGF in the periphery contributes to the maintenance of the thermal hyperalgesia. Because the reversal is so quick, the NGF must be acting directly or indirectly on the peripheral terminals to alter transduction sensitivity. In other words NGF appears to contribute to the peripheral sensitization of high threshold thermoreceptors in a manner that has a short half-life and that requires, therefore, a continuous supply of NGF.

Mechanical hypersensitivity, produced by adjuvant inflammation, is not, unlike thermal sensitivity, immediately reversed by anti-NGF serum (figure 3*b*). Instead a reduction in mechanical sensitivity only manifests 24 h after the anti-NGF administration and becomes more prominent at 48 and 36 h. The delay before an effect of removing peripheral NGF can be detected may well reflect the time that is required for retrograde transport to the DRG, signal transduction, altered transcription and translation and protein/peptide transport.

4. NGF TARGETS IN THE PERIPHERY

There are three trkA expressing targets that could be influenced by an elevation in NGF levels during inflammation; small calibre neuropeptide expressing primary sensory neurons (McMahon *et al.* 1994), postganglionic sympathetic neurons (Smeyne *et al.* 1994) and inflammatory cells (Lomen-Hoerth & Sooter 1995) (figure 4). Each could potentially lead to sensitivity changes. The inflammatory cells could do this by releasing inflammatory mediators on the binding of NGF to trkA (Pearce & Thompson 1986; Bischoff & Dahinden 1992). This seems to occur for mast cells which release the amines histamine and 5-hydroxytryptamine amongst other mediators on NGF-induced degranulation, and depletion of mast cells with the degranulating compound 48/80 attenuates systemic NGF-induced hyperalgesia (Lewin *et al.* 1994). This compound also attenuates inflammatory hyperalgesia but this may be because it reduces the

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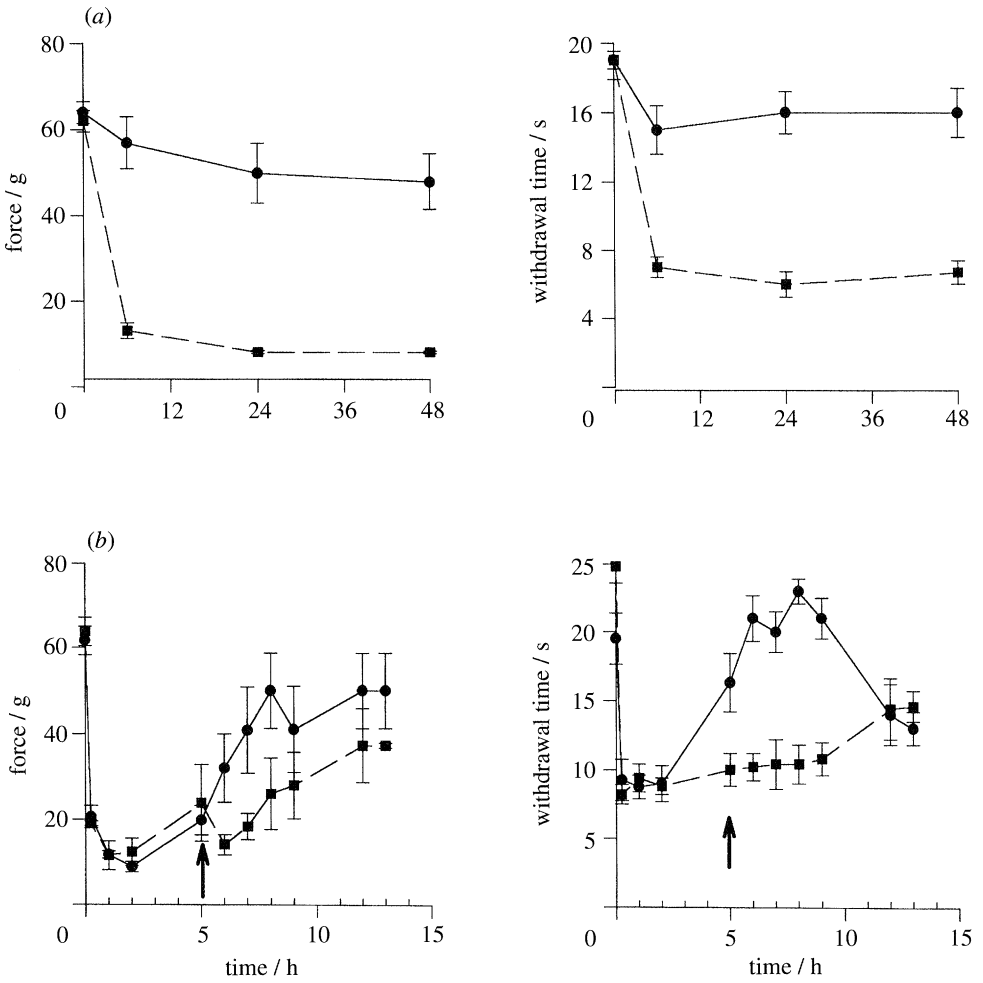

Figure 3. (*a*) Freund's induced inflammation of the hindpaw produces a rapid and marked increase in mechanical (left hand panel) and thermo-sensitivity (right hand panel). This is almost completely prevented by systemic pre-treatment with a specific neutralizing anti-NGF serum. (*b*) When inflammatory hypersensitivity is well established following intraplantar Freund's, administration of anti-NGF at day 5 results within an hour in a highly significant reduction in the thermal hypersensitivity (right hand panel). Mechanical hypersensitivity (left hand panel) is only decreased 24–48 h after the anti-NGF administration. This delay is compatible with an interruption of NGF-mediated changes in transcription in the DRG. Modified from Woolf *et al.* (1994). Circles: anti-NGF + CFA; squares: CFA.

production of NGF by mast cells (C. J. Woolf, unpublished observations).

A role for the postganglionic sympathetic neurons in inflammation has been extensive studied (Green *et al.* 1993) and although the acute effects of NGF appear to be sympathetic-dependent , sympathectomy only produces a very transient reduction or delay in the manifestation of inflammatory hypersensitivity (C. J. Woolf *et al.*, unpublished data). It is unlikely that the sympathetic nervous system, although a prototypic example of an NGF-dependent tissue, is a major target from the inflammatory upregulation of NGF, at least as far as hypersensitivity is concerned.

That leaves the last remaining NGF target: the trkA expressing primary sensory neurons. These cells, almost all of which are CGRP-containing and many of which contain SP (Averill *et al.* 1995), may be affected directly by NGF in two ways. NGF on binding to trkA will necessarily activate its intracellular tyrosine kinase domain which may initiate local changes in the peripheral terminal of the sensory neuron. The tyrosine kinase may directly or indirectly lead to the phosphorylation of membrane proteins including ion channels or transducing molecules which could alter sensitivity. A decrease in K^+ channel or an increase in Na^+ or Ca^{2+} channel activity could all manifest as increased sensitivity. The second way in which NGF interacting with trkA on sensory neurons could alter function is by being internalized and by the retrograde transport of the NGF-trkA complex to the cell body in the DRG where, by changing transcription, it may lead to increased sensitivity.

5. PHENOTYPIC MODIFICATIONS AFTER INFLAMMATION

Acute inflammation has been shown in a number of studies to alter the levels of neuropeptides substance P and CGRP in primary sensory neurons (Noguchi *et al.* 1988; Donnerer *et al.* 1992; Woolf *et al.* 1994; Leslie *et al.* 1995; Safieh-Garabedian *et al.* 1995). The extent to which the elevated peptide levels are due to an

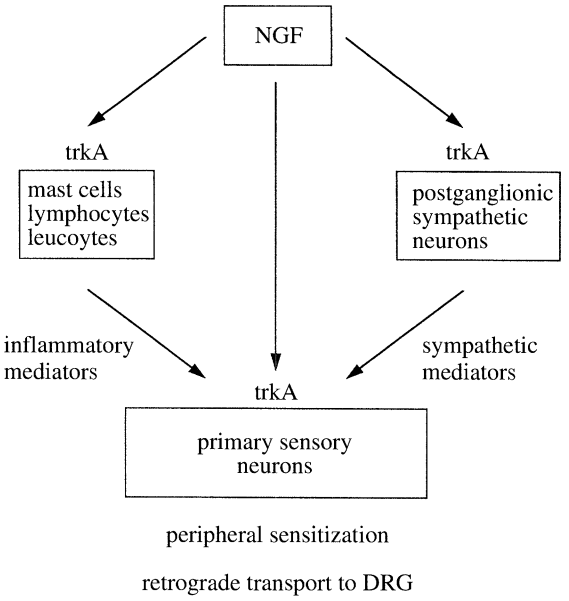

Figure 4. The three trkA expressing targets present in inflamed tissue and the mechanisms by which they may contribute to the hyperalgesic actions of NGF.

increased production of cells which normally express these peptides or to the novel expression in cells which normally do not contain the peptides has not been fully resolved. What is clear though, as illustrated in figure 5, is that the number of DRG cells positive for preprotachykinin A and CRP mRNA increase very substantially, with a greater than twofold increase after an adjuvant-induced inflammation of the hindpaw. Given that approximately 20% of lumbar DRG cells are normally substance P positive using immunohistochemical and in situ techniques, and that the total number of L4 DRG neurons is 16–18000, this means that many more neurons innervating the inflamed paw now express mRNA for preprotachykinin A than normal. Whether all these cells express the peptide is not known but we have found a significant increase in the number of myelinated afferents which are substance P positive after inflammation (in preparation). This indicates that there is a change in phenotype after inflammation. The fact that anti-NGF administration effectively prevents the peptide and mRNA alterations induced by inflammation (figure 5) shows that this neurotrophin is involved.

A new finding from our laboratory is that in addition to alterations in neuropeptides, inflammation also alters the levels of the growth-associated protein GAP-43 (Leslie *et al.* 1995). The time course of the increase in GAP-43 mRNA and preprotachykinin A mRNA are very similar and both are restricted to the dorsal root ganglion innervating the inflamed tissue (figure 5*b*). However, although both Gap-43 and preprotachykinin A mRNA are increased by inflammation in an NGF-dependent fashion, only preprotachykinin A mRNA is increased by the administration of NGF (Leslie *et al.* 1995). This shows that NGF administration does not model the inflammatory state and that while NGF is necessary it is not sufficient for all the phenotypic changes that occur.

6. FUNCTIONAL CONSEQUENCES OF PHENOTYPIC CHANGES

An alteration in the expression of any neuropeptide/protein in primary sensory neurons may potentially modify both peripheral and central terminals. Although the scale of phenotypic changes that occur after inflammation has not been fully investigated yet, the available data shows multiple potential alterations that could lead or contribute to sensory hypersensitivity (figure 6).

Both substance P and CGRP are released from peripheral and central terminals of primary afferents. In the periphery they are vasoactive producing vasodilatation and substance P increases capillary permeability, which together comprise neurogenic inflammation. If NGF during inflammation produces an increase in the peptide production of neuropeptide containing cells and if sensory neurons which do not normally express peptides begin to do so, then it is probable that the neurogenic component of inflammation may be augmented.

Of more significance for the development of hypersensitivity is the central role of the neuropeptides. Substance P acting on NK_1 receptors in the dorsal horn is responsible for the slowest portion the slow synaptic potentials evoked by stimulating C-fibres (Nagy *et al.* 1994) and has a major role in the induction of prolonged excitability increase that occurs after brief C-fibre inputs, the phenomenon of central sensitization (Ma & Woolf 1995). Central sensitization involves a marked increase in the glutamate sensitivity of dorsal horn as a consequence of a protein kinase C-mediated reduction in the normal voltage-dependent magnesium block of the NMDA receptor-ion channel complex at resting membrane potentials (Chen & Huang 1992). Substance P, by binding to the NK_1 G protein-coupled receptor, mobilizes intracellular Ca^{2+} and activates PKC. An increase in substance P release from central terminals coupled with an upregulation of NK_1 receptors in dorsal horn neurons (McCarson & Krause 1994) make it likely that during inflammation the central effects of primary afferent input are greatly amplified. We have electrophysiological evidence that stimuli that are quite innocuous when applied to normal non-inflamed skin begin to evoke a central sensitization-like phenomenon in inflamed animals in an NGF-dependent fashion and that this is due to central excitability changes (Q.-P. Ma & C. J. Woolf, unpublished data). Of particular interest is the involvement of myelinated afferents in the phenomenon. As most myelinated afferents do not express trkA, this raises the question how an NGF-dependent phenotype alteration may occur in such afferents (figure 6). One possibility is that NGF acting on trkA expressing cells results in a paracrine release of signal molecules such as BDNF which acting on trkB-expressing cells changes their phenotype.

The upregulation of GAP-43 during inflammation may relate to structural alterations in the neurons. GAP-43 is a developmentally regulated protein that is re-expressed after nerve injury and is associated with axonal outgrowth (Chong *et al.* 1992). After inflam-

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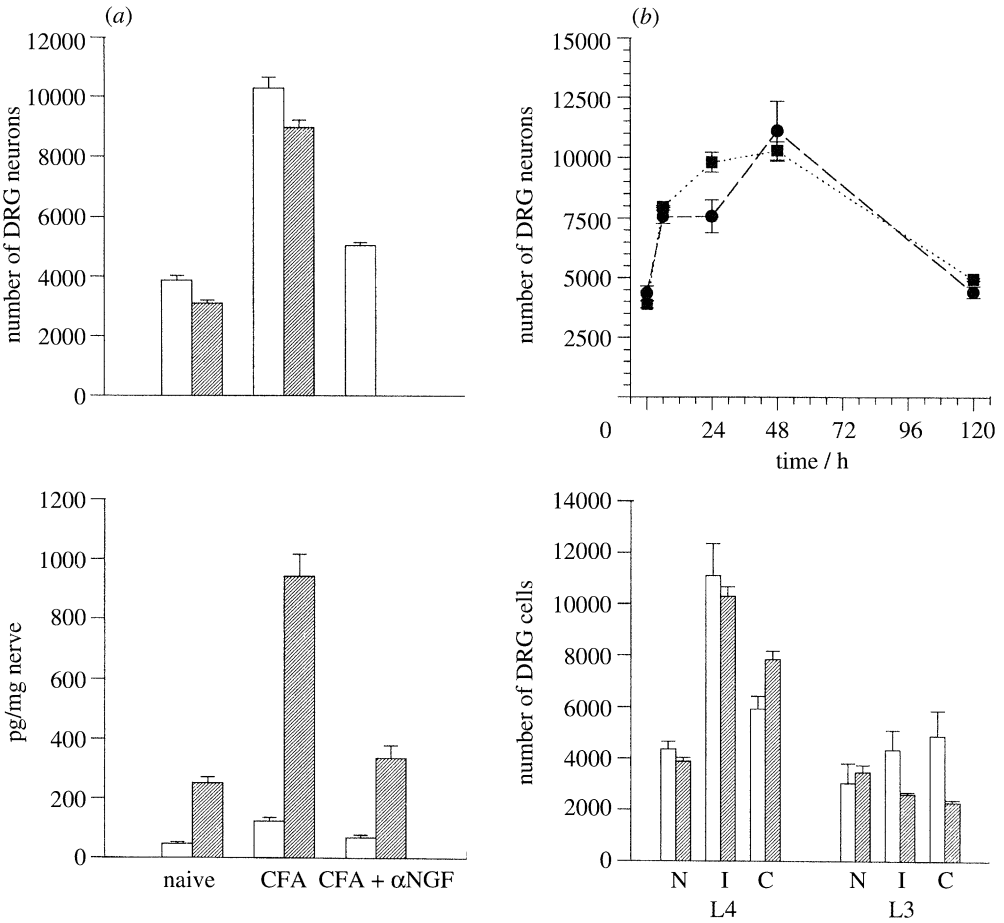

Figure 5. (*a*) Alteration in the expression of preprotachykinin A and CGRP mRNA in the DRG (top panel) and peptide levels in the sciatic nerve (bottom panel), 48 h after inflammation, and prevention of the increases by anti-NGF pre-treatment. Modified from Woolf *et al.* (1994) and Leslie *et al.* (1995). (CGRP mRNA and anti-NGF not tested). Open areas: SP; hatched areas: CGRP. (*b*) Time course of changes in the number of DRG neurons expressing preprotachykinin A and GAP-43 mRNA after induction of an adjuvant inflammation in one hindpaw (top panel). The lower panels show that the increase in expression is limited to that ganglion (L4) innervating the inflamed site. The adjacent ganglion (L3) show no change. N = naive, I = ipsilateral to inflammation, C = contralateral hindpaw. Open areas: SP; hatched areas: GAP-43. Modified from Leslie *et al.* (1995). Circles represent GAP-43; squares represent substance P.

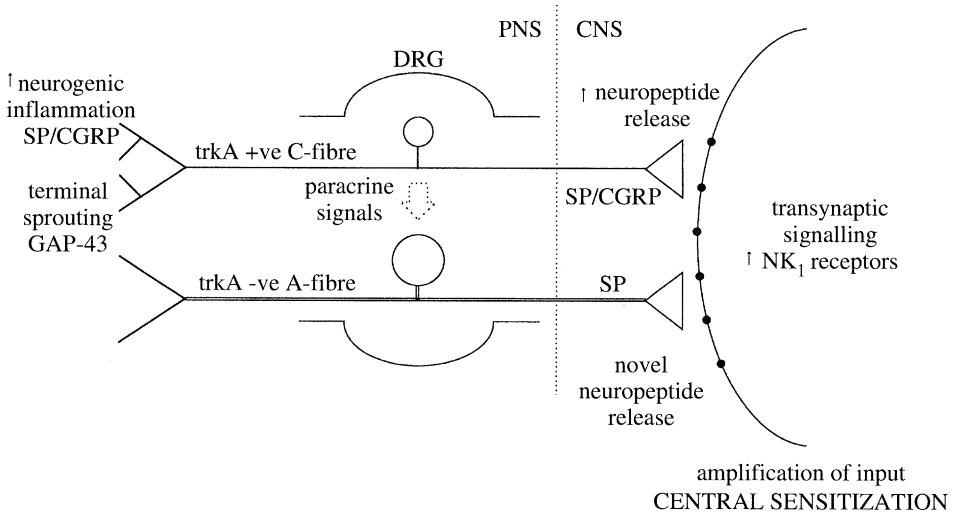

Figure 6. A model illustrating how an NGF-mediated modification in primary sensory neuron phenotype may lead to persistent pain by producing central and peripheral changes in sensory neuron function.(See text).

mation there is both an NGF-mediated increase in expression and the novel appearance of GAP-43 immunoreactive fibres in the epithelium of inflamed skin (Leslie *et al.* 1995). This may indicate a hyperinnervation of the inflamed skin which would amplify inputs to the CNS.

7. CONCLUSIONS

The increase in NGF production during inflammation plays a major role in the production of inflammatory hypersensitivity changes. Some of these changes are the consequence of peripheral sensitization and involve NGF interacting with inflammatory cells, sympathetic neurons and primary sensory neurons to alter transduction sensitivity. An additional contribution is the consequence of an NGF-mediated alteration in the phenotype of sensory neurons. This appears to result in structural changes leading to hyperinnervation, and the upregulation of neuromodulators which act in the spinal cord to increase neuronal excitability and, therefore, amplify incoming inputs. There remains much more to be discovered about the nature of the signals involved and the changes produced in sensory neurons during inflammation but it is clear that inflammatory hypersensitivity is an expression of the plasticity of neurons and that NGF is a key player. Apart from providing an insight into the broader role of this neurotrophin in the adult nervous system, this work also offers novel targets for the treatment of persistent pain.

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