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Neurotrophins and Sensory Neurons: Role in Development, Maintenance and Injury. A Thematic Summary

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Neurotrophins and sensory neurons: role in development, maintenance and injury. A thematic summary

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1. INTRODUCTION

Cells in sensory ganglia have constituted a very productive model system to examine the biology of the neurotrophins. Reasons for this have included the accessibility of the cells and their processes to studies of development, function and plasticity after injury. Despite their apparent homogeneity at the gross level sensory neurons are highly differentiated in their anatomy, physiology, chemistry, etc. Their response to neurotrophins varies among the different cell types which has helped to elucidate the action of these agents. It is the purpose of this brief review to summarize a number of overriding themes that emerge from these studies as discussed at the meeting on *Neurotrophins and sensory neurons: role in development, maintenance and injury*. Reference is made throughout to contributions of the speakers at this meeting whose papers may be consulted for further detail and for the appropriate references.

2. DIFFERENT NEUROTROPHIN FUNCTIONS DURING THE LIFE SPAN OF THE ANIMAL

As recently as 5 years ago work on neurotrophins was dominated by their demonstrated role as a 'survival factor'. These studies were centered largely on the effects of NGF on small diameter neurons, in large part because NGF was the first neurotrophin to be identified. The hypothesis summarizing a large body of work of this type has become known as the 'neurotrophic hypothesis' which states that neuronal survival is dependent on the availability of a target derived factor, the neurotrophic factor. As additional neurotrophins (BDNF, NT-3) became identified they were found to have neurotrophic effects on different classes of DRG cells, particularly the larger ones (see A. M. Davies & W. D. Snider, this volume).

Conventional neurotrophic action is confined to a relatively brief developmental period, in mammals surrounding the time of birth. Recently, numerous studies have identified additional roles for neurotrophins that are operative outside the perinatal period. Thus Kalcheim (this volume) has demonstrated that NT-3 supports the survival of the precursors of a large population of neurons, larger than that expressing *trk*C receptors in the adult, and promotes the migration and differentiation of neural crest precursors into neurons. Antibodies to NT-3 also reduce cell numbers in the DRG when administered during the same early developmental period. However, application of NT-3 to developing sensory ganglia in quail causes a paradoxical reduction in cell number if administered very early (stages 3–6) presumably as a consequence of decreased proliferation (Y. Barde, this volume). Again, the reduction in cell number is not limited to the large cells to which NT-3's neurotrophic action is restricted later in development, and this is consistent with this is a much wider distribution of *trk*C receptors on cells of the DRG at this early stage than later when it is limited to large, presumed proprioceptive, neurons. In general these early actions are exerted by NT-3 and to some extent by BDNF, but not NGF, in keeping perhaps with the older evolutionary history of BDNF and NT-3 compared to NGF. The switch in neurotrophin dependence when the growing axon reaches its target appears to be intrinsically programmed and may represent a strategy to maximize innervation of the target and thus select the final survivors from as large a population as possible (A. M. Davies, this volume).

At later stages neurotrophins exert effects on phenotypic expression of specific sensory properties. Lewin (this volume) reported that exogenous NT-3 causes C-fibres destined to develop into nociceptors to be converted into low threshold mechanoreceptors whose projections are absent from the normal nociceptor projection zone of the spinal cord (lamina II). Similarly, nociceptive afferents with A-delta axons, exhibit an apparent switch in phenotype to that of low threshold D-Hair in preparations deprived of NGF via systemic administration of an NGF antibody (G. R. Lewin, this volume). This switch occurs *after* NGF's neurotrophic action has ended i.e. after postnatal day 2, but only within a relatively brief time window i.e. a critical period. The same treatments also alter the properties of nociceptors with unmyelinated axons somewhat less drastically but these changes may also represent a phenotypic switch. BDNF heterozygotes (BDNF +/−) exhibit specific deficits in the function of SA1 mechanoreceptors and Aδ nociceptors, indicating an important role for this neurotrophin in the

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expression of these phenotypes (G. R. Lewin, this volume). These targets are quite different from those on which they act as a survival factor.

Neurotrophins can also affect function in adults. Again, the best studied example comes from NGF where it has been demonstrated that even a single administration elicits hyperalgesia within a few minutes that lasts for several days (see both S. B. McMahon and C. J. Woolf, this volume). There is now evidence that the acute action involves autonomic neurons (S. B. McMahon, this volume) as well as non-neuronal cells such as mast cells (see C. J. Woolf, this volume). These actions can be described as 'pharmacological', with NGF influencing sensory neurons either directly or via cells with the appropriate high affinity receptors which release transmitters that sensitize or activate sensory neurons. However, there is almost certainly a physiological role for NGF in inflammatory pain as it is upregulated as a consequence of injury, and the hyperalgesic consequences of inflammation can be blocked by administration of neutralizing antibodies to NGF (C. J. Woolf, this volume) or *trk*A-IgG fusion molecules (S. B. McMahon, this volume). Although the knockouts indicate that neurotrophins other than NGF can also influence the properties of sensory receptors (G. R. Lewin, this volume), there is as yet no direct evidence in adults that these neurotrophins or their antibodies have acute effects that can be attributed to their direct action on sensory neurons.

Collectively, these studies indicate that the neurotrophic hypothesis describes only a small part of neurotrophin action on sensory neurons throughout the life span of the animal. Even during development it is clear that neurotrophins have function that exceeds their role in assuring neuronal survival, and beyond that they can influence sensory function in the adult.

3. SPECIFICITY OF NEUROTROPHIN ACTION

With the identification of the high affinity receptors for the neurotrophins (*trk*A/NGF; *trk*B/BDNF, NT-4; *trk*C/NT-3, see R. M. Lindsay, this volume) and the availability of methods such as *in situ* hybridization and immunocytochemistry to localize *trk* function on individual neurons, it has become possible to establish the specific cellular targets of neurotrophins. Parameters used to infer function have included cell size, presence of peptide markers (CGRP, substance P) and projection fields in the spinal cord. These markers are useful indicators but are not absolutely diagnostic. Nonetheless, it has become evident that these receptors are distributed among sensory neurons in adults in a highly ordered fashion-*trk*A on small diameter, peptide containing cells that are nociceptive in function, *trk*C on large, presumptively proprioceptive neurons and *trk*B on cells that are of medium size (H. S. Phillips, this volume). Mice with 'knockouts' of the NGF/*trk*A system or the NT-3/*trk*C system lack peripheral nociceptive or proprioceptive neurons, respectively as demonstrated by a specific absence of projections to laminae I and II or the ventral horn, respectively (W. B. Snider, this volume). Physiological studies in NT3 +/− mice support a further role for NT-3 in assuring the survival of D-Hairs and SA-1 mechanoreceptors (G. R. Lewin, this volume). The role of the BDNF/*trk*B system in dorsal root ganglion development and function is enigmatic at present with suggestions that it is generally co-expressed with *trk*A or *trk*C, at least in adults (H. S. Phillips, this volume) and that it may function as an autocrine/paracrine- derived survival factor for a significant minority of cells in adults (A. L. Acheson, this volume). Mice heterozygous for the BDNF gene (BDNF +/−) exhibit elevation of mechanical threshold of SA1 and mechanical nociceptors (G. R. Lewin, this volume), indicating at least some physiological role for the *trk*B receptor.

However, as implied above from studies of neurotrophin action at different developmental stages, the relation between cell type and neurotrophin is not necessarily a unique one throughout development. In addition, there is evidence that a population of lectin-binding small diameter afferents stops expressing *trk*A during development and expresses no *trk* in the adult (H. S. Phillips, this volume). This accounts for the finding that NGF or *trk*A knockouts yield a greater loss of cells measured in the adult than express *trk*A in the adult (W. B. Snider, this volume). Finally, the relation between the neurotrophins and *trk*s is not completely monogamous with evidence, for example, that NT-3 can serve as a ligand for the *trk*A receptor (R. M. Lindsay, W. D. Snider and G. R. Lewin, this volume).

Another issue related to specificity of action concerns the action of the *trk* receptors in initiating activity in various intracellular signaling pathways that lead to cell differentiation and function (see D. R. Kaplan, this volume). The different *trk* receptors are thought to differ primarily in their binding capacity i.e. whether they bind NGF, BDNF, etc. and this determines which cells are the targets of which neurotrophin (see above). However, evidence in related systems (PC12 cells) suggests that activation of *trk*s by different growth factors can lead to divergent results, e.g. cell proliferation or differentiation. Similar differences are seen in sensory neurons at different stages of development (A. M. Davies, this volume). As the *trk*s are thought to have similar structures, this leads to the question of how receptors whose structure is similar have different cellular actions (D. R. Kaplan, this volume). Suggestions that might account for these differences include the duration of the downstream effect (i.e. whether it is transient or sustained) as well as the possibility that the identity of proteins that are phosphorylated, and thus which intracellular cascade is initiated, depends on which growth factor activates the *trk*.

Thus although there is some specificity in the interaction between neurotrophins and *trk*s, the relations are not absolutely one-to-one. Furthermore, the change in *trk* expression in individual cells during the life span of the animal indicates that the relation between neurotrophins and cells of a particular functional type is undoubtedly not fixed.

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4. SOURCES OF NEUROTROPHINS: ARE THEY DERIVED STRICTLY FROM THE TARGET ORGAN?

The neurotrophic hypothesis implies that neurotrophins are derived from the target organ. Although there is considerable direct evidence for the presence of neurotrophins in skin and muscle (see R. M. Lindsay, this volume), recent data indicate a much broader distribution of these substances in association with the sensory neuron. The cell bodies themselves express the mRNA for neurotrophins indicating the possibility of a paracrine or autocrine effect on sensory neurons, acting perhaps as a survival factor for sensory neurons in adults (A. L. Acheson, this volume). In addition, Schwann cells and fibroblasts in association with the axon can synthesize neurotrophins such as NGF and BDNF after the axon has been damaged, suggesting a potential role in regeneration (P. Richardson, this volume). During early stages of development neurotrophins derived from the neural tube influence the migration of neural crest precursors (C. Kalcheim, this volume).

5. NEUROTROPHIN RECEPTORS: LOW AND HIGH AFFINITY

Neurotrophin receptors are of 2 types, low affinity and high affinity. The high affinity receptors have tyrosine kinase activity and are known as *trk*s (*trk*A, *trk*B or *trk*C) with high affinity binding to NGF, BDNF/NT-4 and NT-3, respectively (see both D. R. Kaplan and R. M. Lindsay, this volume). The ability to identify these immunocytochemically or via *in situ* hybridization has been very valuable in helping to localize potential targets of neurotrophin action. A potential problem in such analysis is the presence of truncated forms of these receptors that might be recognized by the probe but which would be incapable of transducing the neurotrophin stimulus. These truncated receptors might act to bind free neurotrophin molecules and thus act to diminish their action (R. M. Lindsay, this volume).

It has been thought that the low affinity receptor, known as p75, functions in parallel with high affinity receptors, acting to modulate their activity possibly via interference with autophosphorylation. This suggests that the ratio *trk*/p75 rather than the absolute level of either one is the important variable in determining *trk* efficacy (D. R. Kaplan, this volume). Some evidence (e.g. from knockouts) indicates a closer relation to the *trk*A receptor than to the others.

6. NEUROTROPHIN ACTION: TRANSMITTER, CYTOKINE OR HORMONE?

Substances that act as mediators of intercellular communication are often divided into 3 major classes: neurotransmitters, hormones and cytokines. Neurotransmitters are responsible for short distance communication between neurons and consist either of relatively small molecules such as amino acids or classical neurotransmitters such as acetylcholine or the biogenic amines. Peptides also function as neurotransmitters although in a somewhat different manner (see below). Hormones are substances that travel through the blood stream to affect cells at a long distance from the secreting cell. Cytokines are soluble mediators of cell-cell communication that generally act at relatively short distances but larger than the synaptic cleft. These agents that include classes such as the interleukins generally function to signal between cells of the immune system. In this respect neurotrophins act like cytokines and may be especially influential in mediating interactions between the immune and the nervous system (C. J. Woolf, this volume).

Neurotrophins are peptide molecules that bind to specialized receptor molecules, the *trk*s. This bears a resemblance to the general process of synaptic transmission. The action of synaptic transmitters is often differentiated into ionotropic and metabotropic, the former involving direct gating of ionic channels thereby changing current flow (time course in milliseconds) and the latter involving cascades of reactions via intracellular messenger molecules (time course in seconds or minutes). However, the actions of the neurotrophins can be initiated over a wide range of times from minutes to hours or days. The reason is that the products of the growth factor-*trk* receptor interaction can lead to phosphorylation of existing proteins (rapid onset) or synthesis of new ones via neurotrophin action on the gene expression (slow onset) (D. R. Kaplan, this volume). The upregulation of peptides CGRP and substance P in nociceptive sensory neurons that may contribute the long lasting component of hyperalgesia (C. J. Woolf, this volume) is an example of such long term effects. The ability of neurotrophins to promote axon outgrowth or cell survival would represent other examples of these long term effects.

However, these agents, acting as cytokines, have been shown also to have acute effects that may involve the action of non neural cells that can release transmitters which can affect sensory neurons, e.g. effects of NGF on degranulating mast cells thereby sensitizing nociceptors (see S. B. McMahon and C. J. Woolf, this volume). The possibility of direct acute effects on sensory neurons themselves, possibly via metabotropic channels, cannot be discounted at the present time. These acute actions might contraindicate the use of these agents in certain clinical situations where their trophic effects would be desirable (e.g. in preventing degeneration of certain neurons).

Consideration of the neurotrophins as transmitters highlights the fact that we know relatively little about how neurotrophins are released from cells in which they are synthesized. These cells can be but are not typically neurons, e.g., NGF is synthesized and released from mast cells (C. J. Woolf, this volume) or Schwann Cells (P. Richardson, this volume). The nature of the release process as well as the regulation of this release and synthesis of the neurotrophins is not well understood.

7. NEUROTROPHINS AND FUNCTION IN THE ADULT: POSSIBLE CLINICAL USES

The recognition that neurotrophins can act throughout the postnatal life of the animal to influence function indicates the possibility for therapeutic uses of these agents. Experimental studies of axotomized sensory neurons reveals alterations in *trk* and peptide expression that can be at least partially reversed by administration of neurotrophins (P. Richardson, this volume). Furthermore, sensory neurons are severely compromised in experimental models of diabetes (D. R. Tomlinson, this volume) and their targets exhibit changes in neurotrophin levels that are generally consistent with a role for these molecules in the sensory pathology. These changes in neurotrophins and sensory function are largely reversible by administration of insulin. It is suggested that administration of neurotrophins might also help to reverse these sensory neuron deficits. A recent clinical study suggests that a selective impairment of sympathetic adrenergic function might be a consequence of a primary loss of neurotrophin levels in the skin (P. Anand, this volume).

The fact that NGF is hyperalgesic when administered to animals and to humans indicates a potential problem in using this molecule therapeutically to arrest or reverse degenerative processes. However, because NGF is upregulated as a consequence of inflammatory injury (C. J. Woolf, this volume) and because antibodies to NGF can interfere with inflammatory hyperalgesia (C. J. Woolf, this volume), agents that prevent NGF accumulation might have value in alleviating inflammatory hyperalgesia. *Trk*A-IgG fusion molecules have proven experimentally useful in this situation (S. B. McMahon, this volume).

8. EXPERIMENTAL MANIPULATION OF NEUROTROPHIN LEVELS

A number of techniques for manipulating neurotrophin levels were discussed in various contexts. It is important to appreciate that decreases or increases in neurotrophin levels test different components of their interaction with these neurons. Decreased neurotrophin levels test their physiological role; increased levels may speak more to their pharmacological role as in many cases they appear to be present to excess.

(*a*) *Use of neutralizing antibodies*

The use of neutralizing antibodies has the advantage over knockouts in being able to be delivered at precise times so that the effects can be studied as a precise function of development. This has permitted the delineation of a critical period in the action of NGF on the phenotypic expression of nociceptor properties. However, antibodies are large molecules whose free access to their targets is not constant over the life span of the animal. In rats, the blood brain barrier is established only 2–3 weeks after birth and so the administration of antibodies would have decreasing access to the CNS over this period when the effect of neutralizing antibodies directed against neurotrophins is of special interest. Access to target tissues such as the skin may also change during this period and this must be factored into the interpretation of these experiments.

(*b*) *Knockouts*

Mice have been created that are homozygous for deletion of the gene for one of the neurotrophins or the receptors (low or high affinity). Because some of these deletions are lethal, other strains have been made that are heterozygous at these locations. Anatomical and physiological studies have revealed discrete deficits in sensory neuron survival (*trk*A/NGF knockout: nociceptors; *trk*C/NT-3 knockout: proprioceptors) as a consequence of these manipulations (see W. D. Snider, this volume). These findings are largely in agreement with those obtained using *in vitro* methodology i.e. examining which neurons placed in culture during the period of cell death survive when provided with a particular neurotrophin (A. M. Davies, this volume). The agreement between the results obtained *in vitro* where time of exposure to a particular neurotrophin is precisely controlled and at least some other factors are unavailable, and those in knockouts where the action of only a single neurotrophin is totally eliminated throughout development is striking in view of the broad and sometimes conflicting changes that neurotrophins, particularly NT-3 and BDNF, have been found to exert at different stages of development (see above). Thus a fuller understanding of the effects of the knockout depends on determining whether the precursors of the neurons that are found to be missing in the adult are present at the anticipated time, and if so whether in normal numbers. A simple interpretation is that the neurotrophins' role in survival determines which neuronal classes are eliminated by the missing gene without regard for the relative numbers of neurons of different types present as a result of the earlier effects of the knockout.

The deficits seen in the knockouts are often dose dependent in the sense of being substantially greater in the case of animals homozygous for a deletion than in those that are heterozygous (A. M. Davies, this volume). These findings are consistent with the neurotrophic hypothesis in the sense of the availability of the neurotrophin being the limiting factor in neuronal survival. However, the disparity in DRG cell depletion in NT-3 and *trk*C knockouts (*trk*C knockouts lose about 20%; NT-3 knockouts lose about 50%) suggests that NT-3 may have a role additional to ensuring survival of the *trk*C DRG cells. Taken at face value it could suggest that NT-3 acts to support neuronal populations not endowed with the *trk*C receptor. How this would square with the inhibitory effects on proliferation alluded to by Barde (this volume) is not clear.

In this meeting we have also heard evidence that knockouts heterozygous for NT-3 exhibit altered sensitivity of low threshold afferents with Aδ axons (D-Hairs) as well as of slowly adapting Type I receptors (SAI) with Aβ axons (G. R. Lewin, this volume). It is not clear whether this represents survival of particular

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populations of afferents or whether it is the result of a specific functional change in the affected neuron or some important supporting cell.

(*c*) *Fusion molecules with trk receptor sites*

A new technology involves the use of *trk* receptors fused to IgG molecules to act as a circulating target for growth factors thereby preventing them from reaching their normal targets in the appropriate concentration. This has been demonstrated to be useful in reversing inflammatory hyperalgesia (S. B. McMahon, this volume), much as NGF antibodies.

(*d*) *Injections of neurotrophins*

This has been carried out systemically or locally to good effect. The dosages in systemic administration tend to be rather high (1 mg kg^{-1}) and in the case of NGF some adverse immunological responses have been noted. Reports from human subjects injected with NGF indicate substantial sensory abnormalities, most clearly identified with nociception (see S. B. McMahon and C. J. Woolf, this volume).

(*e*) *Transgenic constructs using promoter genes to control tissue of expression*

A recent technique involving the fusion of the NGF gene or its antisense to a keratin promoter has enabled selective neurotrophin expression in the skin. These mice have been shown to experience sensory abnormalities similar to those exhibited by animals subjected to systemic injection of the same neurotrophin (NGF).

9. CONCLUDING REMARK

In closing it seems appropriate to present a tabulation of unresolved issues and general conclusions that can serve as a basis for further study. These are necessarily incomplete but they express the author's perspective on where things stand at the present time.

(*a*) *Unresolved issues*

1. In view of the highly complex neurotrophin actions during development why are knockouts so precise in their phenotype?

2. What is the significance of the apparently opposite actions of NT-3 on neuronal proliferation?

3. How is the cellular release of neurotrophins regulated?

4. What is the function of having different *trk* species on individual cells? Do these promote different cellular functions? This is particularly relevant for *trk*B which is often expressed in conjunction with either *trk*A or *trk*C. A related question is whether BDNF and NT-4 are required for survival of specific cells as NGF and NT-3 appear to be.

5. Is NGF unique among neurotrophins in having a physiological role in the adult?

6. Are NT-3 and BDNF unique in having roles in the earliest stages of development?

7. Is the activation of *trk*B by both NT-4 and by BDNF purposeful or simply an evolutionary accident?

(*b*) *General conclusions*

1. Neurotrophin action is not limited to assuring neuronal survival. They also affect proliferation, differentiation, phenotypic expression and recovery after injury.

2. Neurotrophins are not derived only from target tissue. They are synthesized in *trk*-expressing neurons and can affect neurons locally including themselves via paracrine or autocrine actions.

3. The relation between neurotrophins and *trk*s is not a unique one. The interaction can be modified by the low affinity receptor (p75) and individual *trk*s can be activated by more than one ligand.

4. *Trk* expression is not restricted to the same neuronal populations throughout the life of the animal which means that the relation between neurotrophins and individual cells may not be fixed.

5. Neurotrophin/*trk* interactions resemble neurotransmitter/ receptor metabotropic interactions. The results can be initiated relatively quickly (phosphorylation of currently synthesized propteins) or more slowly due to changes in gene expression.

6. Neurotrophins can have acute effects that resemble those produced by drugs.

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