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Control of Neuronal Survival by Neurotrophins

Abstract

Neurotrophins are related secretory proteins that control cell survival in the nervous system. All can prevent programmed cell death by binding to specific cell surface receptors belonging to a family of tyrosine kinase receptors. As these receptors are expressed in subgroups of developing neurons, interference with the function of these receptors or of their ligands leads to selective neuronal deficits in the nervous system. All neurotrophins also bind to another receptor designated the neurotrophin receptor p75.

This member of the tumor necrosis factor receptor family can be activated by nerve growth factor, leading to the death of neurons in the developing nervous system. Thus, the neurotrophin nerve growth factor controls cell numbers in opposite ways by its ability to activate 2 different receptors.

Introduction

In multicellular organisms, programmed cell death is now widely recognised as an important mechanism contributing, like cell division, to the control of cell numbers (Raff, 1992). In the developing nervous system, the death of cells can already be observed during the earliest stages of development, in proliferating neuroepithelia such as the neural tube or the retina (Glücksmann, 1951; Cuadros and Rios, 1988; Homma et al., 1994). Later, when identifiable groups of neurons cease to divide and begin to make contact with their target cells, the extent of cell death can be better appreciated by counting the total number of cells before and after target innervation. During this phase, neuronal death can be readily quantified, and in a variety of neuronal populations, losses of 50% or more have been reported (for review, see Oppenheim, 1991). However, the real extent of cell death during early neurogenesis is still difficult to appreciate quantitatively. The fate of individual cells cannot be traced on a large scale, and dying cells are rapidly eliminated. By contrast, in the nematode *Caenorhabditis elegans*, the total number of cells is so small that it is possible to monitor the fate of every cell during development. These quantitative studies have revealed that out of the 1090 originally somatic cells generated, 131 die, 105 of which being neurons (for review see Ellis et al., 1991).

Studying the mechanisms controlling developmental cell death is of special significance in the nervous system, as unlike other cells, neurons typically become postmitotic early and never divide subsequently. In vertebrates, several molecules regulating the survival of neurons have been identified, including in particular a small gene family known as the neurotrophins, which consist of 4 members in mouse and human. These genes encode very basic, secretory proteins named nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin3 (NT3) and neurotrophin4/5 (NT4/5). The neurotrophins are non-covalently bound dimers, and even though the existence of soluble monomers has been demonstrated, these monomers spontaneously reform dimers (Kolbeck et al., 1994). Crystal structure data indicate that the neurotrophins have very similar, elongated shapes, with the monomers forming a large hydrophobic interface (McDonald et al., 1991; Robinson et al., 1995). The known biological properties of the 4 neurotrophins can be accounted for by their ability to bind to 2 distinct receptors, one designated the neurotrophin receptor p75 ($p75^{NTR}$), the other consisting of a group of closely related receptor kinases referred to as the *trk*s.

The interaction of all neurotrophins with their *trk* receptors prevents neuronal death, whereas recent results indicate that the interaction of one of them, NGF, with $p75^{NTR}$ causes cell death.

II. Neurotrophins and their Tyrosine Kinase Receptors

A. Binding of Neurotrophins to the trk Receptors

A group of 3 closely related receptor tyrosine kinases, referred to as *trk*s, has been identified in higher vertebrates. The *trk*s bind neurotrophins specifically with nanomolar affinities (for review, see Barbacid, 1994). NGF binds to *trk*A, BDNF and NT4/5 to *trk*B and NT3 to *trk*C. Neurotrophin binding induces dimerisation of the receptors, and the activation of their kinase domains results in tyrosine phosphorylation (for review, see Kaplan and Stephens, 1994). Two recent findings help to explain why BDNF and NT4/5 have biological effects which are not always identical, even though both bind to *trk*B with identical affinities. An extracellular splice variant of *trk*B has been discovered which preferentially interacts with BDNF over NT4/5 (Strohmaier et al., 1996). Also, the participation of $p75^{NTR}$ in a (still hypothetical) p75/*trk*B complex seems to be more critical for NT4/5 binding, including retrograde transport, than it is for BDNF (Curtis et al., 1995; Rydén et al., 1995).

B. Activation of trk Receptors prevents Neuronal Death

The generation of mouse mutants with functional deletions in the *trk*A, B and C genes has allowed comparisons between the phenotype of such animals and that of mice with deleted neurotrophin genes (for reviews, see Barbacid, 1994; Snider, 1994). This work led to the conclusion that the *trk*s are essential for transducing the neuronal survival activity of neurotrophins. Thus for example, when the peripheral sympathetic or sensory system of *trk*A -/- animals is compared with that of NGF -/-

animals, the same neuronal subpopulations are absent (Snider, 1994). This suggests that in order to prevent programmed cell death, activation of tyrosine kinase receptors by neurotrophins is necessary.

How the activation of receptor tyrosine kinases interferes with programmed cell death is still not well understood. Presumably, a phosphorylation cascade prevents at some point the activation of intracellular proteases. Indeed, genetic experiments using *C. elegans*, made possible because of the small number of cells in this nematode (see above), led to the crucial observation that a null mutation in the *ced-3* gene blocks programmed cell death, and that *ced-3* acts cell autonomously (Yuan and Horvitz, 1990). Subsequent work revealed that *ced-3* encodes a protease related to a previously identified vetebrate protease with specificity for aspartate residues (Yuan et al., 1993). Inhibition of such proteases prevents programmed cell death in neurons (Gagliardini et al., 1994). Also, injections into the cytoplasm of cultured neurons of plasmids encoding *bcl-2* block programmed cell death in neurons (Garcia et al., 1992). *Bcl-2* was discovered because of a chromosomal translocation in human (positioning *bcl-2* under the inappropriate control of an immunoglobulin promoter), resulting in excesses of B-cells (and of lymphoma), explained by a lack of cell death (Tsujimoto and Croce, 1986). Significantly, the nematode gene *ced-9*, the function of which is necessary to prevent programmed cell death, encodes a protein clearly related to bcl-2 (Hengartner and Horvitz, 1994; for review, see Horvitz et al., 1994). Thus, it appears that the intracellular mechanisms controlling programmed cell death in neurons are basically similar to those uncovered in *C. elegans* using a genetic approach.

C. Specificity of trk Expression and of Neurotrophin Action

The discovery that members of the *trk* family are functional receptors for the neurotrophins has been important and useful, as the pattern of expression of these genes in the nervous system helps to predict which neuronal populations are likely to respond to which neurotrophin (Tessarollo et al., 1993).

The specificity of the neurotrophin-*trk* system is especially well documented in the peripheral sensory system. In particular, it has been possible to generate animals lacking specific types of sensory inputs such as those involved in nociception or proprioception (for a recent review, see Lewin and Barde, 1996). This can be achieved either by injecting antibodies neutralising the neurotrophins or their *trk* receptors, or by deleting the corresponding genes. To a large extent, these results can be explained by the fact that many small, unmyelinated sensory neurons and axons express *trk*A, but not *trk*C, which is expressed by large sensory neurons, including the myelinated Ia afferents contacting ventral horn motoneurons (reviewed in Snider, 1996; Lewin and Barde, 1996). The specificity of the resulting phenotype is likely to be of considerable use to understand the molecular diversity of sensory neurons involved in the perception of various stimuli. In the CNS, while the expression of *trk* receptors (at least in rodents) is less obviously correlated with the survival of specific neuronal populations than is the case in the PNS, the pattern of *trk* expression remains a very useful predictor of neurotrophins' actions, such as for example effects on gene expression or dendritic arborisation (Ip et al., 1993; McAllister et al., 1995).

D. Significance of Neurotrophins in the Developing CNS

In chick embryos, antibody deprivation experiments have indicated that neurotrophins also control the development of CNS neurons (Bovolenta et al., 1996). In this regard, the retina has been a particularly useful structure to study, both because of its rapid development (millions of cells being generated over only few days), and its accessibility to experimental manipulations. Many cells in the neural retina express the catalytic NT3 receptor *trk*C, and some $p75^{NTR}$ (see below). Also, NT3 is present in the retina, mostly in the pigmented epithelium early in development. Acute deprivation of NT3 results in profound modifications affecting the development of most cell types (Bovolenta et al., 1996). In particular, as soon as the optic nerve forms, about half of the axons are missing in the optic nerve of antibody-treated embryos. This deficit does not reflect a developmental retardation, as it persists for as long as the optic nerves have been examined. Also, a general reduction in the size of all retinal layers indicates that NT3 is an essential retinal signal influencing the fate of many cells. Detailed examination of such embryos has revealed that NT3 must be more than an essential survival factor for developing retinal cells. Indeed, NT3 seems to play a role as a differentiation factor, needed to take retinal neuroblasts out of the cell cycle. Thus in the absence of NT3, cell division has been shown to be abnormally high in the retina (Bovolenta et al., 1996).

It thus appears that neurotrophins are essential survival factors for a variety of developing vertebrate neurons, that they need to activate tyrosine kinases to prevent programmed cell death, and that at least NT3 is also an essential differentiation factor early in the development of CNS neurons.

III. The Neurotrophin Receptor $P75^{NTR}$

A. Neurotrophin Binding to $p75^{NTR}$, a Member of a Gene Family

When $p75^{NTR}$ is expressed in fibroblastic cell lines, it binds all neurotrophins with similar, nanomolar affinities, though when binding parameters are examined in detail, different kinetics and degrees of positive co-operativity are observed (Rodríguez-Tébar et al., 1990, 1992; for review and discussion, see Bothwell, 1995; Dechant and Barde, 1997). This suggests that differential conformational changes are induced by neurotrophin binding. Indeed, the results of recent functional experiments have indicated that specific effects can be elicited by the neurotrophins through $p75^{NTR}$ (see below).

$P75^{NTR}$ was the first member to be isolated of a still growing family of structurally related, non-catalytic receptors (for review, see Chao, 1994). These include in particular the TNF receptors I and II (TNFRI and TNFRII), and the Fas antigen (also referred to as Apo-1 or CD95). These receptors are characterised by an extracellular domain containing usually 3 or 4 repeated cysteine-rich subdomains. However, their intracellular sequences are not obviously related and lack any motifs providing clues as to the type of biochemical reactions they may catalyse. The only clear intracellular

homology is a sequence of about 60 amino acids in Fas and TNFRI. This segment has been coined the 'death domain', as these 2 receptors have clearly been shown to induce programmed cell death in a variety of cell types (Itoh and Nagata, 1993; Tartaglia et al., 1993; Nagata and Golstein, 1995). This death domain also shows some amino acid identities with the sequence of the *reaper* gene (White et al., 1996), known to cause programmed cell death in *Drosophila* (Golstein et al., 1995).

B. Functional Consequences of Neurotrophin Binding to p75^{NTR} in vitro

One of the functions of p75^{NTR} that has received much attention is its ability to increase the affinity of NGF binding to *trk*A, and to form a high affinity receptor on cells expressing both *trk*A and p75^{NTR} (see in particular Mahadeo et al., 1994). But many cells in the nervous system and elsewhere express p75^{NTR} and not *trk*A, which has a comparatively restricted pattern of expression. So an intriguing question has been, for a long time, whether neurotrophin binding to p75^{NTR} on cells not expressing catalytic forms of *trk*-receptors would have any measurable biochemical or biological consequences.

One of the first clear answer came from experiments performed with p75^{NTR}-expressing T9 glioma cells, where neurotrophin binding was shown to transiently activate a sphingomyelinase, resulting in the production of the lipid second messenger ceramide (Dobrowsky et al., 1994; Dobrowsky et al., 1995). Evidence that p75^{NTR} can signal in non-transformed cells was subsequently provided using cultured rat Schwann cells, and the addition of NGF was shown to lead to the translocation of the transcription activator NF-κB to the nucleus (Carter et al., 1996). This activation of NF-κB occurs through the binding of NGF to p75^{NTR}, as Schwann cells isolated from p75^{NTR} -/- mice did not show translocation of NF-κB. Interestingly, neither BDNF nor NT3 activate NF-κB (Carter et al., 1996). While the biochemistry of this selectivity is not yet understood, substantial differences in the p75^{NTR} binding parameters have been noted when NGF, BDNF and NT-3 are compared (see above). Also, differences were observed in the circular dichroism spectra of p75^{NTR} when mixed with each of the neurotrophins, suggesting different conformational changes accompanying binding (Timm et al., 1994). Finally, in recent experiments, p75^{NTR} was shown to form high affinity, specific binding sites for NT3 when expressed in neurons, as opposed to fibroblastic cell lines (Dechant et al., 1997). This suggests that p75^{NTR} may associate with cytoplasmic proteins, opening the possibility that the cellular context in which this receptor is expressed could dramatically affect the ability of p75^{NTR} to signal (see also Dechant and Barde, 1997 for review).

Recent work with cultured oligodendrocytes indicates that binding of NGF to p75^{NTR} by NGF activates jun kinase and initiates programmed cell death (Casaccia-Bonnefil et al., 1996). Again, this effect could not be seen with NT3 or BDNF. When ceramide production was measured following exposure of oligodendrocytes to the various neurotrophins, only NGF, but neither BDNF nor NT3, triggered a sustained release of ceramide. Taken together, these results suggest that, as with the activation of NF-κB, only NGF is able to activate p75^{NTR} to cause cell death.

C. *Functional Consequences of Neurotrophin Binding to p75^{NTR}* in vivo

It is clear that the expression of p75^{NTR} is, as such, not sufficient to predict an activation of NF-κB or cell death by NGF. As with the other receptors of the p75^{NTR} family, interaction with cytoplasmic proteins is likely to be required to transduce any effects of ligand binding, and until such interactors have been identified, it will be difficult to predict signalling by p75^{NTR} merely by looking at the tissue distribution of this receptor. This raises the important question of whether or not any of the observations with cultured cells is relevant to the situation *in vivo*, and recent experiments indicate that this seems to be the case. Early in development in the avian retina, substantial cell death is observed, mostly in the central retina (Cuadros and Rios, 1988). It has been hypothesized that this might relate to the formation of the optic nerve, as all the axons of the retinal ganglion cells converge to the central retina to form the optic nerve (Cuadros and Rios, 1988). In addition, previous work has indicated that p75^{NTR} is expressed by many cells in the central avian retina (Von Bartheld et al., 1991). As expression of the NGF gene is detected very early in the chick retina in the absence of *trk*A expression (Frade et al., 1996), it was of interest to see if removal of NGF would decrease cell death. Such turned out to be the case, and to a degree indicating that most early cell death is actually mediated by NGF (Frade et al., 1996). In the absence of *trk*A, p75^{NTR} is *a priori* likely to be the receptor mediating the death action of NGF. Direct evidence for the involvement of p75^{NTR} could be provided by the intraocular administration of antibodies blocking the binding of NGF to p75^{NTR}, which prevented cell death much like the administration of antibodies to NGF (Frade et al., 1996).

D. *Targeted Deletion of the p75^{NTR} Gene*

The gene coding for p75^{NTR} has been deleted in the third exon, coding for 3 of the 4 cysteine-rich repeats (Lee et al., 1992). These mice present sensory deficits in the peripheral nervous system (Lee et al., 1992), as well as subtle signs of hypoinnervation in the peripheral sympathetic system (Lee et al., 1994). This result fits well with the notion that when co-expressed with trkA, p75^{NTR} increases the affinity of this receptor, enhancing in vivo the detection of low levels of NGF. Interestingly, these mice also show increased numbers of cholinergic forebrain neurons, as a *trk*A-negative population of such neurons which is normally eliminated in wild-type animals fails to disappear in mutant animals (Van der Zee et al., 1996). This result is in agreement with the suggestion that NGF can eliminate neurons through activation of p75^{NTR} during normal development, in cells not expressing *trk*A (see above). However, compared with any of the *trk*-deficient animals, the phenotype of the p75^{NTR} is considerably less spectacular. In particular, these mice not only survive the mutation, but they are also able to breed. Given the increasing number of similarities in the biological effects of NGF mediated by p75^{NTR} and those of TNF mediated by TNFRs, it might be more useful to compare the p75^{NTR} mouse mutant with those lacking TNFRs, rather than *trk* receptors. Mice lacking either TNFR receptor show a phenotype that becomes obvious when they are challenged with infectious agents, such as *Listeria monocytogenesis*. Also, these mice are actually more resistant to endotoxic shock than control mice (Pfeffer et al., 1993; Rothe et al., 1993).

As NGF has the potential to kill neurons expressing $p75^{NTR}$, it will be interesting to see if such mechanisms are also used in physio-pathological situations. For example, motor neurons are known to dramatically upregulate $p75^{NTR}$ after axotomy (Raivich and Kreutzberg, 1987; Yan and Johnson, Jr. 1988; Ernfors et al., 1989), and the administration of NGF has been show to increase the death of axotomised motor neurons (Miyata et al., 1986; Sendtner et al., 1992), while it can be prevented by the administration of BDNF (Sendtner et al., 1992).

Conclusions

With regard to neuronal survival, the physiological significance of the neurotrophins is well established. Their ability to prevent programmed cell death by activating specific receptor kinases accounts for their long known survival effects, both *in vitro* and *in vivo*. The specificity of many of their actions on various subpopulations of neurons can be explained by the patterns of expression of their specific *trk* receptors. But it is now apparent that the best known neurotrophin -NGF- can also cause cell death through its apparently unique ability to activate $p75^{NTR}$, when this receptor is expressed on cells not expressing *trk*A. How widespread this killing action of NGF is will be interesting to investigate in future experiments. Also, the molecular mechanisms linking NGF and $p75^{NTR}$, but not the other neurotrophins, with the cell death machinery need to be understood.

While this review deals with the regulation of neuronal survival during development, this should not be taken to imply that the control of programmed cell death represents the only biological function of the neurotrophins. In the CNS in particular, there is mounting evidence that neurotrophins change the morphology of neurons, and regulate the growth of dendrites and axonal terminals (see for example Cohen-Cory and Fraser, 1995; McAllister et al., 1995). As neurotrophins are synthesised in, and released by, CNS neurons as a function of neurotransmitter input, they might trigger morphological changes in relation with neuronal activity (for review, see Thoenen, 1995). So far, evidence has been mostly presented for positive or enhancing actions of neurotrophins on neuronal morphology (Snider and Lichtman, 1996). However, the theoretical possibility also exists that regressive events might be initiated by the activation of $p75^{NTR}$ by NGF. While purely speculative at this stage, one could envisage that local retraction of dendritic or axonal branches utilises mechanisms similar to those involved in cell death.

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