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Neurotrophins: structural relatedness and receptor interactions

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SUMMARY

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) are structurally related proteins that allow the survival of specific populations of embryonic vertebrate neurons. The primary structure of these neurotrophins, deduced from their nucleotide sequences, indicates that all three are synthesized in the form of precursor proteins presumably allowing for appropriate folding, including the formation of disulphide bridges, cleavage and secretion. While no information is yet available on the 3-dimensional structures of the neurotrophins, results from binding studies using the three neurotrophins as ligands indicate that their receptors do recognize similarities, as well as differences, between them. High-affinity receptors, that presumably mediate the biological response, as well as low-affinity receptors are present on neurons responsive to the neurotrophins. Whereas a large excess of heterologous ligand is needed to reduce binding of a particular neurotrophin to its high-affinity receptor, the same concentration of homologous or heterologous ligand similarly reduce the binding of any of the three neurotrophins to the low-affinity receptor. For all three, the low-affinity receptor appears to be the already characterized NGF low-affinity receptor that seems to be an integral part of the high-affinity receptor complexes. These results suggest that the regulation of neuronal survival by target cells can, in part, be explained by the release from these cells of limiting quantities of the structurally related neurotrophins, each being recognized by a specific high-affinity receptor complex located on the nerve terminals of the responsive neurons.

1. INTRODUCTION

During the early development of the vertebrate nervous system, neurons are produced in excess numbers, followed by the elimination of a substantial proportion of the neurons initially present. This naturally occurring neuronal death occurs at the time when the axon terminals reach the vicinity of their target cells (for review, see Cowan *et al.* (1984)), and can be regarded as a mechanism allowing quantitative adjustments to be made between two structures that, at first, are not connected. This is an interesting phase of neuronal development to study, not least because at no other time during the lifespan of higher vertebrates are the changes in neuronal numbers so dramatic and rapid. In addition, there is good evidence to suggest that the target cells play a major role in the establishment of their own innervation. Ablation experiments show that the reduction in size of a presumptive target field results in a dramatic increase in the extent of neuronal death on the corresponding neuronal population. Conversely, artificially increasing the target field appreciably reduces neuronal cell death (Cowan *et al.* 1984). Molecules have thus been postulated to be synthesized in the target cells, released in limiting amounts by these cells and to be required by the developing neurons for their survival (for recent reviews, see Purves (1988) and Barde (1989)). Specific receptors are thought to be present on the incoming nerve terminals and to detect the presence of these molecules in the vicinity of the target cells. Neurons whose axonal terminals have detected threshold levels of such molecules are selected for survival. Some structural features of three related molecules, referred to as 'neurotrophins', thought to be relevant in this biological context are discussed, as well as some of their binding characteristics on their receptors.

2. NEUROTROPHINS

Until now, three molecules are known to support the survival of embryonic neurons and to show considerable structural relatedness (figure 1): nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). All have been shown to support the survival of selected neuronal populations *in vitro* (for a recent review, see Bailey *et al.* (1990)). In all three cases, the complete primary structure has been deduced from genomic DNA or

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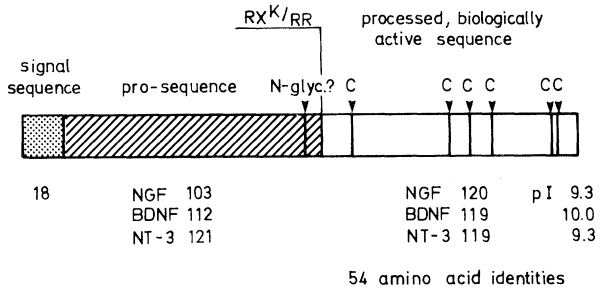

Figure 1. Mouse neurotrophin. Schematic representation of the common structural features found in NGF, BDNF and NT-3 deduced from their nucleotide sequences. (K, Lysine; R, Arginine; C, Cysteine.)

cDNA clones (Scott *et al.* 1983; Ullrich *et al.* 1983; Leibrock *et al.* 1989; Ernfors *et al.* 1990; Hohn *et al.* 1990; Kaisho *et al.* 1990; Maisonpierre *et al.* 1990; Rosenthal *et al.* 1990). Several features common to the three neurotrophins are summarized in figure 1. Particularly striking is the structural relatedness of the biologically active, carboxyterminal half of these molecules with 50% amino-acid identities, including all six cysteine residues located at identical positions. The exact arrangement of the three disulphide bridges present in NGF and BDNF (and assumed to be also present in NT-3) is known only for NGF, and is 1–4, 2–5 and 3–6. For BDNF, preliminary data from tryptic cleavage followed by microsequencing of some of the resulting fragments indicate a 1–4 linkage in BDNF as well (F. Lottspeich & Y.-A. Barde, unpublished results). From protein chemistry data, it appears that mouse NGF (at least as purified from the mouse submandibular gland) lacks the last two terminal amino acids (Angeletti & Bradshaw 1971; Scott *et al.* 1983; Ullrich *et al.* 1983). Comparison between data on the amino acid composition of BDNF purified from pig brain and its cDNA sequence (Leibrock *et al.* 1989) suggests that some carboxyterminal processing also occurs in BDNF, and that the last three amino acids are lacking in the protein isolated from pig brain (Y.-A. Barde & F. Lottspeich, unpublished results). As to the quaternary structure, mouse NGF is known to exist under non-denaturing conditions as a homodimer (Bothwell & Shooter 1977). Less is known about BDNF and NT-3, although their chromatographic behaviour also suggests that these two proteins can form dimers.

The availability of cDNA probes for the three neurotrophins has allowed studies to be conducted on their sites of gene expression. While each of the three genes has its own pattern of expression (see, for example, Korsching *et al.* (1985); Ernfors *et al.* (1990); Hofer *et al.* (1990); Hohn *et al.* (1990); Maisonpierre *et al.* (1990); Rosenthal *et al.* (1990)), some common features have been observed. The three genes are expressed not only during development, but also in the adult, and usually at higher levels than during the early stages of neurogenesis. These genes are expressed not only in non-CNS tissues, where NGF and NT-3 predominate, but also in the CNS, where the expression of the BDNF gene is highest. It is intriguing to see that in fact in rodents, the highest levels of CNS expression of all three neurotrophins is in the hippocampus. Importantly, the cellular source of gene expression has also been identified and in the intact CNS seems to be confined to neurons (Ayer-Lelièvre *et al.* 1988; Hofer *et al.* 1990). At present the physiological relevance of these findings has not been shown directly. It is unlikely that the main or only function of these neurotrophins in the adult brain will be that of keeping adult CNS neurons alive. More likely speculations are the maintenance of differentiated functions, the regulation of neurotransmitters levels, as well as the modulation of local sprouting. In this context, the localization of these mRNA to neurons is of particular significance and it will be important to see how the levels of these proteins and of their release is modulated by the neurotransmitters acting on the neurons synthesizing these neurotrophins.

3. NEUROTROPHIN RECEPTORS

In view of the structural similarities between the neurotrophins, the question arises as to the extent to which their receptors can discriminate between them. Binding studies using radioiodinated NGF and BDNF indicate that on responsive neurons, two classes of BDNF and NGF receptors can be distinguished on the basis of their dissociation constants (K_d): high-affinity receptors ($K_d \approx 10^{-11}$ M) and low-affinity receptors ($K_d \approx 10^{-9}$ M) (Sutter *et al.* 1979; Rodríguez-Tébar & Barde 1988). Preliminary studies performed with NT-3 also show the presence of two classes of receptors on responsive neurons. As the characteristics of these two classes of receptors markedly differ in terms of heterologous ligand discrimination, they will be discussed separately.

(*a*) *Low-affinity receptor*

Recent studies suggest that the low-affinity NGF-receptor is also a low-affinity receptor for BDNF (Rodríguez-Tébar *et al.* 1990). This NGF receptor gene has been cloned (Johnson *et al.* 1986; Radeke *et al.* 1987) and is a single-copy gene in all species tested so far (Hempstead *et al.* 1988; Large *et al.* 1989). The studies of heterologous ligand discrimination by the low-affinity receptor was facilitated by the availability of a fibroblast cell line (PCNA) stably transfected with this gene, and expressing large number of receptors, which bind NGF with characteristics corresponding to the low-affinity NGF-receptor on neurons (Radeke *et al.* 1987). Like with NGF, while no binding of either BDNF or NT-3 was detected on the parental cell line (not transfected with the NGF low-affinity receptor gene), BDNF as well as NT-3 binding was observed after transfection. The addition of unlabelled BDNF and NT-3 prevented the binding of NGF to the PCNA cells to the same extent as the addition of unlabelled NGF did, indicating that the NGF receptor gene codes for a cell surface protein able to bind the three ligands with identical affinity. However, kinetic experiments revealed differences between the three ligands. When the rate of dissociation from the low-affinity receptor was measured, it was found to be different for all three ligands. The fastest rate of dissociation was observed

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with NGF, followed by NT-3, with that of BDNF being considerably slower than the other two. In addition, the saturation curves also showed differences: unlike with NGF, the binding of BDNF to the low-affinity receptor was found to be sigmoidal, indicative of positive cooperativity. This was also observed, though less pronounced, with NT-3. It is thus clear that although the low-affinity NGF receptor binds the three neurotrophins, this receptor also recognizes differences between the three ligands. This finding is important as it suggests that the ligand-induced conformational changes of the common receptor are different (see below). The view that there might be just one low-affinity neurotrophin receptor is not only not in contradiction, but, if anything, supported by studies on the distribution of what has been regarded so far as the NGF low-affinity receptor. Indeed, several studies using *in situ* hybridization with probes detecting the low-affinity NGF-receptor or immunohistochemical studies with a monoclonal antibody recognizing this receptor indicate that this molecule is present on many NGF-non responsive cells including BDNF or NT-3 responsive neurons (Raivich *et al.* 1985; Buck *et al.* 1988; Ernfors *et al.* 1988; Yan & Johnson 1988; Yan *et al.* 1988). In fact, this receptor is found also on cells not responding to any of the three neurotrophins known so far, such as in particular the spinal cord motoneurons (Yan *et al.* 1988; Ernfors *et al.* 1989). Assuming that this receptor recognizes structural features that neurotrophins have in common, and taking its expression as an indication for the existence of a putative ligand, it can be predicted that the neurotrophin family is composed of more members than the three characterized so far.

(*b*) *High affinity receptor*

High-affinity neurotrophin receptors exist on responsive neurons, each binding one of the three ligands with affinities in the order of 10^{-11} M. Unlike with the low-affinity receptor, competition studies indicate that the high-affinity binding of any neurotrophin will only be displaced by a large excess of heterologous ligand (up to 1000-fold). As it appears that unlike with the low-affinity receptor, there exists a good correlation between the presence of high-affinity receptors and biological response, the key to the understanding of this exquisite ligand discrimination as well of the message transduction is in the identification of the molecular components resulting in high-affinity binding. One of these molecules is in all probability the common neurotrophin or NGF low-affinity receptor. A convincing demonstration of this comes from experiments performed by using PC12 cells, a cell line that responds to NGF and displays low- as well as high-affinity NGF receptors (Landreth & Shooter 1980). There exists a PC12 mutant cell line that has lost its ability to bind NGF. When the gene coding for the low-affinity receptor is introduced into these cells, both low- and high-affinity binding is restored (Hempstead *et al.* 1989). This result also indicates that the component(s) resulting in high-affinity binding is incapable of significant binding in the absence of the low-affinity receptor. That, in addition, other molecular components are involved in high-affinity binding is indicated by cross-linking experiments by using labelled NGF (Hosang & Shooter 1985; Hosang & Shooter 1987).

While there are a number of examples in the recent literature of receptors composed of more than one subunit, in particular the integrins and the receptors to many interleukins, the neurotrophin receptors seem to be so far unique in that very probably, one component, the low-affinity receptor, is the same for all. One possible model suggested by the experiments described here is that binding of the neurotrophins to the low-affinity NGF-receptor induces a conformational change of this receptor that is specific and different for each neurotrophin. This change then triggers the specific association with other molecules resulting in a receptor complex with considerably higher affinity and specificity, and able to trigger the biological response. However, other models are also compatible with the data, such as pre-existing high-affinity receptor complexes. Clearly, attention will now focus on the molecular characterization of these unknown elements conferring high-affinity and selective binding, as they are likely to give clues to the nature of the intracellular signals generated upon the binding of neurotrophins to their cell surface receptors and resulting in neuronal survival.

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