Molecular Interactions Modulating Neuronal Survival and Growth

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ABSTRACT: The extracellular environment of the neuron provides a heterogeneous milieu of survival and growth modulating molecular species subserving regulatory signals that operate in development, meditate activity-dependent enduring changes in synaptic connectivity, and promote or inhibit survival and axonal regeneration following insult. Parallel distributed processing networks in neurons, activated by these molecular species, can likely be recruited selectively to serve specific needs of the organism.

RÉSUMÉ: Interactions moléculaires modulant la survie et la croissance neuronale. L'environnement du neurone fournit un milieu hétérogène d'espèces moléculaires modulant la survie et la croissance, favorisant les signaux régulateurs qui agissent sur le développement, qui servent de médiateurs produisant des changements persistants, dépendants de l'activité, dans la connectivité synaptique et favorisent ou inhibent la survie et la régénération axonale à la suite d'une agression. Des réseaux de traitement de l'information, distribués en parallèle dans les neurons, peuvent vraisemblablement être recrutés sélectivement pour remplir certains besoins spécifiques de l'organisme lorsqu'ils sont activés par ces espèces moléculaires.

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Perhaps the two greatest challenges of contemporary neurobiology are the elucidation of the identity and mode of action of those critical biological processes that underlie neural cell injury and curtail or enhance regeneration, and the manipulation of neural responses that can contribute to recovery following damage to the central nervous system.

There now exists a body of compelling experimental data that refutes accepted dogmas that injury to the mature mammalian CNS is irreversible and that regeneration does not occur. With respect to neuronal cells of the CNS, different populations have the intrinsic capacity to initiate and sustain extensive axonal regrowth that can restore functional reconnectivity with distant targets. These neurons, like others in the peripheral nervous system, appear to be responsive to molecular species in the axonal environment that can inhibit or enhance regeneration. Furthermore, injured neurons, in response to environmental cues, have the capacity to reorganize neuronal circuitry in a functionally appropriate manner as a result of sprouting of new processes from their axons.

The thrust of this brief review is to describe some of those molecular species in the axonal environment that influence survival of neurons and regenerative and sprouting responses of axons. The text will highlight recent contributions of the authors to this endeavor, but the reader is directed to comprehensive reviews of the subject matter.

Molecular Interactions Subserving Neuron Survival and Modulation of Process Formation

There is increasing evidence that two broad categories of molecular species contribute to neuron survival and process elongation or growth inhibition by virtue of interactions with neurons singly and as complexes. These two categories are (1) immobilized molecular species of the extraneuronal milieu (extracellular matrix and cell surfaces in juxtaposition to the axon) that promote or inhibit adhesion and neurite extension by local interactions at the growth cone; and (2) diffusible factors that exert effects following axonal uptake and, in some cases, retrograde axonal transport to the cell body. Examples of immobilized species that promote adhesion and neurite growth are laminin, fibronectin, proteoglycans, and collagen. Two glycoproteins of molecular mass 250 kDa and 35 kDa have been implicated in inhibition of neurite growth.[1] The best characterized diffusible molecules are members of the Nerve Growth Factor family, which include NGF, BDNF, and NT3 (reviewed in 2). Other neurotrophic factors, such as ciliary neurotrophic factor (CNTF) and the fibroblast growth factor family (FGF), do not appear to be retrogradely transported, and FGF may exert its effects as a complex with proteoglycan.[2] In addition, a number of neurotransmitters have been implicated in neurite growth and growth inhibition (reviewed in 3).

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The cell sources of these molecular species have not been completely elucidated, but there is emerging evidence that both paracrine and autocrine mechanisms may be acting. Paracrine sources of factors that support or inhibit neurite growth include non-neuronal targets of innervation, support cells of peripheral nerve (Schwann cells and endoneurial fibroblasts), and the glial elements of the CNS. There is also evidence for autocrine production of NGF[4] and of immobilized species such as proteoglycans (PG's),[5,6] in addition to the neurotransmitters that have been implicated in both promotion and inhibition of neurite growth.[3]

While the molecular species that support or inhibit neurite growth derive from many cells types in the nervous system, cell specificity of response to the factors relates to the expression of appropriate receptors for these molecular species on the neuronal cell surface (Table 1). Neuronal receptors for immobilized factors in the extracellular milieu have been partially characterized. A family of receptors known as integrins are widely distributed on cells and interact with specific domains on laminin, fibronectin, and cytotactin (reviewed in 7, and in 8). Laminin also possesses binding sites for heparan sulphate PG's (HSPG's)[8] that form part of the neuronal cell membrane. Cell adhesion molecules (CAM's) have heparin binding domains that can interact with HSPG in the extraneuronal millieu,[9,10] and cell-cell interactions can be mediated by Ca^{++}-independent homotypic and heterotypic interactions between CAM's (reviewed in 11). A group of Ca^{++}-dependent CAM's known as N-cadherins have also been implicated in cell-cell interactions in the nervous system.[12] Cell surface chondroitin sulphate PG's (CPSG's) on neurons interact with the specific glial cell surface glycoprotein, cytotactin.[13] At least two families of cell surface enzyme systems on neurons appear to be involved in neurite growth; these include proteases[14-16] and glycosyltransferases.[17, 18]

Neurons also possess receptors for the NGF family of molecules, as well as for FGF,[2] and neurotransmitter effects on neurons are mediated by specific receptor/ion channel complexes on cell bodies and processes.[3]

The regulation of extraneuronal molecules that support or inhibit neurite growth and their cell surface receptors following insult to the nervous system are only now beginning to be elucidated. In this regard, the influence of inflammatory cells and soluble mediators of inflammation on neurons and non-neuronal cells in the vicinity of injury adds further complexity to the understanding of the molecular interactions occurring in juxtaposition to injured axons.

Table 1: Molecular interactions regulating neuron process growth

	Cell Surface	Extraneuronal Milieu	Reference
Positive			
	Integrin α/β heterodimers	laminin fibronectin cyotactin	7
	Cell adhesion molecules (CAM's)	homotypic & heterotypic interactions	
	Ca++-independent (N-CAM NgCAM, etc..)	HSPG	5, 6, 9, 10
	Ca++-dependent (N-Cadherins)		7, 12
	Proteoglycans (PG's)		
	heparin sulphate PG	laminin CAM's	5,6 5, 6, 9, 10
	chondroitin sulphate PG	cyotactin	13
	Enzymes		
	proteases	serine protease inhibitors	14, 15, 16
	glycosyltransferases	laminin HSPG's type IV collagen	17, 18
	NGF family receptors	NGF, BDNF, NT3	2, 43
	FGF receptors	FGF	2
	Neurotransmitter receptors	glutamate somatostatin	3
Negative			
	Uncharacterized receptors	250 kDa glcoprotein 35 kDa glycoprotein	1
	Neurotransmitter receptors	acetylochloine dopamine	3

Nerve Growth Factor - Influences on Neuron Survival and Axon Sprouting

Because of its ready availability, this 118 amino acid homodimeric protein has long been known to promote survival and to accelerate differentiation of responsive neurons. The protein and its mRNA are localized to target regions of innervation and in support cells in juxtaposition to responsive axons.[2] NGF undergoes retrograde axonal transport to cell bodies of receptor-bearing neurons[19,20] and is taken up into the cell by virtue of receptor-mediated endocytosis.[2] The presence of a highly conserved PEST sequence[21] on the cytoplasmic domain of the NGF receptor provides evidence for the rapid turnover of this cell surface receptor. The mechanism of action of NGF remains to be elucidated clearly, but there is increasing evidence that activation of guanine nucleotide systems may be involved.[22-24]

The survival effects of NGF are manifest in the central nervous system (CNS) and in the peripheral nervous system (PNS). Cholinergic neurons of the basal forebrain are endowed with NGF receptors[25] and can be rescued following axotomy by pharmacological doses of NGF.[26] Endogenous NGF appears to play a role in maintenance and survival of this group of neurons since atrophy and loss of phenotype of intact forebrain cholinergic neurons occurs in the presence of antibody to NGF.[27]

Recently, observations on the effects of NGF on survival and growth of adult mammalian sensory neurons have provided new insights into the regulatory role of this protein. Adult mammalian sensory neurons that contain calcitonin gene-related peptide (CGRP) and substance P also bear high-affinity receptors for NGF.[28] Following axotomy, NGF receptor-bearing cells atrophy and lose receptors for NGF, and retrograde axonal transport of NGF decreases.[29] Administration of pharmacological doses of NGF can rescue the majority of the affected neurons, reversing atrophy and reconstituting the density of NGF receptors,[29] suggesting that NGF can not only promote neuron survival but also regulate the expression of its receptor.

The sprouting of intact sensory axons into skin fields denervated of axons that modulate mechanoreceptor and nociceptive

input is regulated by NGF. Diamond and colleagues[30] have observed that administration of antibody to NGF retards sprouting of intact sensory axons into denervated fields of skin. However, regeneration of sensory axons to restore original fields of innervation is not influenced by NGF, since antibody to NGF had no effect on the rate of functional recovery based on a regenerative response.

The response to peripheral nerve axotomy in the non-neuronal cellular elements of nerve suggests a close regulatory interaction between axon and ensheathing cells. Following axotomy there is a prompt rise in NGF protein and NGF mRNA in ensheathing cells distal to the axotomy and in the region of axonal sprouting. These increases are maintained in the presence of invading macrophages and at least one of the soluble mediators of inflammation - interleukin-1.[31]

Based on these recent observations, one might conclude that, while axonal contact with sources of supply regulates NGF expression and promotes the integrity of intact and lesioned responsive neurons, the protein is not required for the regenerative response if the integrity of the neuron is preserved. That NGF promotes sprouting from intact sensory axons perhaps speaks more eloquently to a role for this protein in plasticity than in functional recovery following insult. Indeed, hippocampal neurons in the CA fields and dentate gyrus are rich in NGF mRNA[4] relative to surrounding glial elements and receive cholinergic input from NGF-responsive neurons in the septal forebrain via the septohippocampal pathway. Since plastic changes in hippocampus involve pyramidal neurons, it remains conceivable that activity-dependent enduring changes in synaptic function in hippocampus, which are associated with growth-dependent changes in cytoskeletal elements,[32,33] might in part involve sprouting of intact septophippocampal cholinergic projections under the control of NGF released from pyramidal neurons.

The observations that activity-dependent changes in neurons are associated with biochemical and morphological evidence of neuronal growth[32-35] highlight the role of neurotransmitters and their receptors in neurite growth and growth inhibition (Table 1). The best characterized neurotransmitter-mediated effects on neurite growth are those related to activation of the NMDA subtype of glutamate receptors. Activation of NMDA receptors is implicated in the phenomenon of long-term potentation (LTP) in mammalian hippocampus,[3,36] and glutamate and NMDA promote neurite growth by responsive neurons *in vitro*.[3]

The Interaction of Axons with Immobilized Molecular Species - Recent Observations

The observation that NGF may be involved less in regenerative axonal responses than in sprouting responses perhaps highlights the function of immobilized molecular species of the extraneuronal milieu in regeneration. It is likely that the faithfulness of the regenerative response that facilitates appropriate connectivity relates in part to guidance pathways provided by the cellular and acellular matrices traversed by regenerating axons. Additionally, guidance may involve pathway preference or the relationship between concentrations of growth-promoting and growth-inhibiting factors in the milieu of regenerating axons.

The regenerating axon sees a heterogeneous milieu that contains molecular species that promote or inhibit adhesion and process extension (Table 1). A number of individual molecules of the extracellular milieu contain more than one domain involved in adhesion and process formation. For example, cell adhesion molecules on support cells provide homotypic or heterotypic binding sites for CAM's on the surface of the axon,[11] but also have sites that can interact with axonal HSPG's.[9,10] Axons can interact with laminin domains involved in adhesion by virtue of receptors known as integrins.[7] However, axons can also interact with laminin by virtue of interactions between axonal HSPG's and heparin binding domains of laminin.[5,6,8] Cytotactin of glial origin interacts with neuronal integrins[7] and also CSPG's.[13]. Finally, neuronal cell surface enzymes of the glycosyltransferase family can interact with appropriate acceptor domains of oligosaccharides on laminin and on the core proteins of HSPG's.[7,18]

In recent studies roles for both heparin domains[5,6] and lactosaminoglycan-type oligosaccharides on laminin[17,18] in neurite formation have been demonstrated. Cell surface HSPG's bind to laminin by virtue of interactions between heparin binding domains on laminin and glycosaminoglycans of this PG. Furthermore, the axonal HSPG's that function in neurite growth appear to bind the axonal surface by non-covalent interactions that also involve glycosaminoglycan (GAG) residues. Thus, HSPG's may function in neurite growth by non-covalent bridging between axonal binding domains and laminin binding domains for GAG residues. These observations add to the heterogenity of interactions that are available to regenerating axons; axons with unoccupied HSPG binding domains might interact with HSPG's immobilized in the extraaxonal milieu, and HSPG's immobilized on axonal binding domains could interact with unoccupied HSPG binding domains on heparin binding molecules in the extraneuronal milieu, such as CAM's and laminin.

Constitutive expression and release of HSPG's and CSPG's by regenerating neurons *in vitro* have been confirmed.[5,6] *In vivo*, both expression and othograde axonal transport of HSPG's and CSPG's are regulated by molecular signals that are activated during regenerative responses in the goldfish retinotectal projection.[37,38] The regulatory signals operate at the level of gene expression[39] and very likely at the level of post-translational processing,[38,40] but phases of regeneration (axonal growth and arborization/synaptogenesis) appear to be distinguishable at the level of post-translational processing of the HSPG's and CSPG's.[38,40]

The neurite-promoting function of axonal galactosyltransferases with laminin involves an interaction between the integral, trypsin-labile enzyme on the cell surface with appropriate lactosaminoglycan-type oligosaccharides of glycoconjugates of the extraneuronal milieu.[17] These substrates are found on laminin, HSPG's and collagen IV, but are not displayed by fibronectin. Following N-glycosidase treatment of laminin, the influence of surface GalTase can no longer be observed, suggesting that the appropriate oligosaccharides are N-linked to sites of glycosylation on laminin polypeptides.[17] These observations extend the catalogue of enzyme-based systems that can promote neurite growth[14-16] and suggest that neurons have the capacity to modulate the intact or injured extraneuronal environ-

ment directly by post-translational processing. Such a mechanism may begin to address the molecular basis of faithful regeneration and connectivity following insult in certain systems.

Conclusion

Neurite growth is subserved by the actions of a highly motile growth cone which samples the cellular and acellular milieu that it encounters. Extension and retraction of microspikes and the movement of filopodia and lamellopodia of the growth cone indicate alternating adhesion and disadhesion phenomena.[41] Rates and direction of neurite growth are determined by hierarchies and gradients of adhesion forces. Diffusible growth-promoting factors may also contribute to the milieu of growth by ensuring the integrity of the neuron and possibly by providing gradients.

The array of macromolecular structures on the growth cone (receptors) and within the extraneuronal milieu (ligands) underscore the heterogeneity of mechanisms that might subserve growth cone motility and advancement. While parallel distributed processing networks[42] might operate to promote neurite extension, it seems clear that those regulatory signals that are involved in neurite extension, whether in growth, regrowth, or during plastic responses, can select from among a group of ligand-receptor interactions.

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