BIOLOGICAL ACTIONS MEDIATED BY MEMBRANE RECEPTORS

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Summary - The peptide hormone nerve growth factor serves several different roles on sensory and sympathetic neurons. It is required for their survival and growth and for the development of differentiated properties including neurite outgrowth and neurotransmitter enzyme induction. Each function is initiated by interaction of the hormone with specific cell surface receptors either on the perikaryon or in the periphery and some of those interactions result in internalization of the hormone. Two different affinities of specific receptors exist. Fractional occupancy of the higher affinity receptor is sufficient to mediate neurite outgrowth while occupancy of both these receptors and the ones of lower affinity are required for enzyme induction. The receptor interactions lead to internalization of nerve growth factor and in a pheochromocytoma cell two intracellular pathways are seen. The first leads to degradation in the lysosomes and the second pathway which occurs later delivers the hormone intact to the nucleus. Nuclear accumulation correlates with the cells' capacity to initiate or regenerate neurites.

An excellent example of the extraordinary diversity and specificity of membrane receptors is found in the nervous system. Nerve growth factor (NGF) is the insulin-like peptide hormone

found in significant quantities in the submaxillary gland of the male mouse and the prostate gland of the guinea pig (18). It exerts profound effects on two types of nerve cells, sympathetic and some sensory neurons (9). Both of these neuronal populations depend on NGF for their survival and growth during embryonic development as evidenced by the inhibitory effects of NGF antibodies *in vivo* and by the requirement for NGF in cultures of these neurons. Growing axons from sympathetic neurons are attracted to sources of NGF. Growth fails if the target organ is removed or if the axons are cut but is restored if exogenous NGF is supplied (5). These phenomena reflect the ability of the growing or mature axon to selectively sequester NGF and carry it by retrograde flow to the interior of the cell body. The existence of this highly specific pathway for NGF and its persistence for the life time of sympathetic and sensory neurons have been amply documented (18). One characteristic response of adrenergic neurons to the retrogradely transported NGF is the selective induction of tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH), rate-limiting enzymes in the biosynthesis of the adrenergic transmitter norepinephrine. Impairment of the retrograde flow of NGF during development by, for example, injection of NGF antibodies causes significant decreases in TH and DBH levels as it also does in the adult if NGF antibodies are generated in the animal by injection of NGF (10).

The induction of TH and DBH *in vitro* requires much higher concentrations of NGF than does the stimulation of neurite outgrowth (7) and as a consequence these two effects of NGF can be readily dissociated. Enzyme induction does not require ongoing RNA synthesis suggesting that this selective regulation occurs at the transcriptional level (13). The NGF-induced stimulation

(regeneration) of neurite outgrowth from explanted sympathetic ganglia is also RNA synthesis independent (12). However the initiation of neurite outgrowth from the PC12 clonal cell line of a rat pheochromocytoma requires transcription (1). As with the primary neurons regeneration of neurites from PC12 cells previously exposed to NGF and from which the neurites have been removed is again independent of new RNA synthesis.

The NGF receptors. The mechanism of action of NGF begins with its interaction with specific cell surface receptors on the responsive cells. Receptors with two affinities of binding have been described on both sensory and sympathetic neurons (17). The fact that the binding of NGF to each of these two receptors is influenced to the same extent when the biological activity of NGF is chemically modified suggests that the receptors share a common molecular form (2). Interestingly the data on the retrograde flow of NGF in sympathetic neurons also lead to the conclusion that it is mediated by two types of receptors, one of high affinity and low capacity and the other of lower affinity and higher capacity (3). The concentrations of NGF required to initiate neurite outgrowth correspond formally to fractional occupancy of the high affinity receptors while those for the induction of TH and DBH correspond to essential complete occupancy of these receptors as well as significant occupancy of low affinity receptors. Further high affinity receptors are only found on neurons and on sensory neurons the high affinity state only appears at the embryonic age at which NGF induces neurite outgrowth (17). Correspondingly the number of NGF receptors on sensory neurons decrease markedly at the embryonic age when the cell ceases to respond to NGF by neurite outgrowth (6). On the other hand receptor numbers and the ratio of high to low affinity receptors on sympathetic neurons

remain nearly constant throughout embryonic life in keeping with their known responsiveness at all embryonic ages. Kinetic data show that the low affinity receptor is not generated from the high affinity by a process of negative cooperativity (17). For example, when neurons are preloaded at concentrations of ^{125}I-NGF sufficient to occupy both types of receptor the subsequent dissociation curves show two rates, one characteristic for each receptor, even when a large excess of unlabeled NGF is added to initiate dissociation. The unlabeled NGF fails to convert all the receptors to the low affinity state as it would if negative cooperativity were a factor. On the other hand the naive PC12 cells possess only the low affinity NGF receptor and it is the binding of NGF which induces a change to the high affinity state (8). Although the molecular basis of this ligand-induced conversion is not known it precedes and may accelerate internalization of NGF into the PC12 cells.

It is unlikely that a single second messenger such as cAMP generated by the NGF-receptor interaction can account for the multiplicity of NGF effects, although this has been argued on the basis that dibutyryl cAMP can mimic some of the responses of PC12 cells to NGF (14). However this agent does not mimic all the effects of NGF although it does act synergistically with respect to stimulation of RNA synthesis and neurite outgrowth and with respect to the latter it does act to overcome the dependency of the NGF-induced neurite outgrowth on RNA synthesis (4). Clearly cAMP can play a role in the mechanism and it would be of considerable interest to identify the substrates for the cAMP dependent protein kinases in PC12 cells. It should also be emphasized that since agents which increase intracellular cAMP do not increase TH levels that cAMP probably is not involved in

this aspect of NGF action (11).

Internalization and messengers. The retrograde flow of NGF from effector organs demonstrates that NGF is internalized by sympathetic and sensory neurites. A variety of approaches have also shown that receptor bound NGF on PC12 cell bodies is internalized in vesicles and a comparison of the down regulation of high and low affinity receptor bound NGF suggests that the internalization is mediated by the high affinity receptors. The intracellular localization of the NGF internalized via the cell bodies of PC12 cells and by retrograde flow in sympathetic neurons in initially similar since the NGF appears in lysosomal structures (15,16). However in sympathetic neurons the NGF remains intact while in PC12 cells it is rapidly degraded. The process of degradation does not in itself give rise to intracellular messengers because PC12 cells will still initiate neurite outgrowth when lysosomal degradation is inhibited. After the period of lysosomal degradation intact NGF appears in the nucleus of the cell. Pulse-chase experiments show that the commitment of the cell to initiate neurite outgrowth correlates with the amount of NGF which accumulates in the nucleus. The ability of these cells to regenerate neurites also depends on nuclear NGF. How the nuclear interaction produces messengers which mediate neurite outgrowth remains an interesting question.

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THE INSULIN RECEPTOR

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The plasma membrane of the cell serves to provide anatomical limits for the functions of the cell while providing ready access into the cell of metabolites required by the cell for normal activity. It has been known for many years that passage into the cell, and from the cell, of nutritive and breakdown metabolites is governed by highly complex systems of diffusion and energy dependent transport that may be specific for individual or related groups of molecules. In recent years the plasma membrane has also been found to be endowed with highly developed cognitive powers in terms of recognizing signals from the extracellular milieu that regulate the metabolic functions of the cell. This capability to receive signals is now known to be accomplished by complex proteins or glycoproteins that are integral units of the cell membrane. Among the most important regulatory signals received by cells are those transmitted through the endocrine system.

Among the most extensively studied receptors for protein hormones is the insulin receptor. It has been found in a variety of cells that are known targets of insulin action including hepatocytes, adipocytes, cells of skeletal and cardiac muscle and placenta, erythrocyte precursors and erythrocytes, monocytes and vascular endothelial cells. A variety of tumor cells also bear insulin receptors in their plasma membranes including lymphoid tumors and erythroleukemic cells. Fibroblasts growing in tissue culture bind insulin and this binding is enhanced if they assume the phenotypic functions of adipocytes (3T3 cells).

It is abundantly clear that the insulin receptors are in a state of continuous dynamic flux. Following exposure to high concentrations of insulin, a sharp diminution in the number of receptors occurs. This phenomenon of mediation of receptor binding by insulin itself is referred to as "down regulation" of insulin receptors. It has been shown to occur in vitro in lymphoblastic cells and fibroblasts. In vivo, a diminution of binding occurs in subjects with hyperinsulinism such as is found with insulinoma. Conversely, when insulin deficiency is induced in animals with agents such as streptozotocin, increased receptor binding is found in cells such as hepatocytes.