

**SIMPOSIO 15.
TROPIC FACTORS SIGNALING IN THE
NERVOUS SYSTEM**

MEDICINA (Buenos Aires) 2004; 64 (Supl. II): 63-65

**P75 NEUROTROPHIN RECEPTOR TRAFFICKING AND SIGNALING IN THE NEURONAL
ENDOCYTOTIC PATHWAY**

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The nerve growth factor (NGF) family of neurotrophin binds two classes of cell-surface receptor, trk receptor tyrosine kinases and the shared p75 receptor. Rapid internalization and retrograde trafficking of neurotrophin-trk complexes have been demonstrated in a number of systems and thought to transmit trophic signals from terminals to neuronal cell bodies. In contrast, internalization and trafficking of neurotrophin-p75 complexes are not well understood. We have demonstrated internalization of p75 with a rather slower kinetic compared to transferrin and trks in PC12 cells and hippocampal cells overexpressing p75 with a SFV1 system. We have shown that p75 internalizes in the recycling endosome and becomes associated with signaling molecules, suggesting that p75 may signal through the endocytic pathway. Such endosomes may participate in retrograde signaling from the tip of the axon to the cell body, as it has been shown for trks. Recently, it was demonstrated that p75 extracellular domain undergoes proteo-

lytic cleavage by PMA-inducible membrane metalloproteinase releasing a soluble extracellular domain. The later enzymatic activity is followed by cleavage within the transmembrane domain of p75 by the gamma-secretase releasing a soluble C-terminal fragment with signaling capabilities. We are studying the proteolytic processing of p75 in PC12 and hippocampal neurons during the endocytic pathway. We have found that p75 is cleaved in both cell types in a ligand dependent manner. Preliminary results suggest that trk activation might be necessary for p75 proteolytic processing. PC12 cells treated with PMA and Compound E (inhibitor of gamma-secretase), accumulate a membrane bound C-terminal fragment (CTF) in early/recycling endosomes suggesting that endosomes are the membrane organelle where p75 is subject to cleavage. All together, these results suggest that in neurons the endocytic pathways might be an important platform for p75 neurotrophin receptor signaling and function.

DEVELOPING RETINA: TGF-BETA CAME THE SECOND?

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Trophic factors are essential during retinal development, triggering signalling cascades for survival, differentiation, and maturation. They also seem to play a key role in retinal regeneration and could be critical in retinal transplantation, where some treatment limitations could clearly be trophic. Retinal cells die when they fail to receive greater exposure than the minimal trophic threshold required, and in some cases this death can be reversed by trophic neuroprotection. Neurotrophins (NGF, BDNF, NT-3, NT-4/5) and their receptors (the Trks and p75) are present and active in the retina of several species and have been identified as important molecules of eye development. In keeping with the general concept of

trophic stimulation, several members of the transforming growth factor family (TGF- β) seem to be seriously involved in the same actions as neurotrophins on retinal cells. The corresponding receptors are expressed during development and work simultaneously with neurotrophins. GDNF—distant member of the TGF- β family—promotes survival and differentiation, and also prevents apoptosis in rat retina during in vitro development. GDNF or Müller cells (possibly producing GDNF in retina) can protect photoreceptors from cell death and has also been demonstrated to have a physiological effect of active molecule on developing neurons from explanted retina. Do these molecules from different families acting directly on

cell survival and indirectly on apoptosis, operate at different times, or do they act together coordinately and synchronously, complementing each other's actions?

The involvement of multiple trophic molecules from different families provides a complex but clear understanding that the simultaneous action of trophins could differ-

entially support the neuronal types or synergism observed in specific retinal neurons (e.g. ganglion cells, amacrine, bipolar, horizontal). This differential growth facilitates the final retinal network, and multifactorial stimulation could also be regarded as a potentially protective mechanism in the developing retina.

ROLE OF THE P75 NEUROTROPHIN RECEPTOR IN MEDIATING NEURONAL APOPTOSIS IN THE CNS

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Neurotrophins support neuronal survival and differentiation via Trk receptors, yet can also induce cell death via the p75 receptor. In fact, the p75 neurotrophin receptor is expressed in many different cell types in the nervous system, and can mediate a variety of different cellular functions, including neuronal survival, cell death and neurite outgrowth. To mediate these different functions, the p75 receptor participates in activating different intracellular signaling pathways. Since the p75 receptor has no enzymatic activity, signaling appears to be transduced by recruiting specific binding proteins to interact with the receptor. Many proteins have been identified that can interact with the p75 intracellular domain, however which of these proteins mediates specific functions in different cell types has not been elucidated.

We have been interested in understanding the many roles of the p75 neurotrophin receptor in the CNS. In par-

ticular, hippocampal neurons express the p75 NTR during development and in the adult after injury. Neurotrophins elicit apoptosis in hippocampal neurons expressing p75, both in culture and in vivo after a seizure. We have been investigating the mechanisms by which this receptor signals neurons to undergo apoptosis. We have demonstrated that the signaling pathway by which p75 NTR induces apoptosis is different from that of other «classic» death receptors, activating the intrinsic rather than the extrinsic caspase pathway. We are continuing to investigate the mechanisms of p75 signaling by examining the different intracellular proteins that bind to this receptor in different cell types and in response to different ligands. Our goal is to gain further understanding of the mechanisms by which neurotrophins and their receptors play a multiplicity of roles in the nervous system.

NOVEL GDNF SIGNALING PATHWAYS REGULATING NEURONAL PRECURSOR DIFFERENTIATION AND MIGRATION

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Glial cell line-derived neurotrophic factor (GDNF) was originally discovered by its ability to promote the survival of ventral midbrain dopaminergic neurons. Four members are known in the family including GDNF, Neurturin (NTN), Persephin (PSP) and Artemin (ART). Each member of the GDNF ligand family binds specifically to one of four structurally related glycosyl-phosphatidyl inositol (GPI)-anchored receptors named GFR α 1-4 (GDNF family receptor alpha) 1 to 4, respectively. Intracellular signaling is mediated by the association of this ligand/receptor com-

plex with the RET tyrosine kinase, a transmembrane protein originally discovered as an oncogene (1, 2). In addition to RET, GDNF and GFR α 1 can also associate with transmembrane isoforms of the neural cell adhesion molecule NCAM, at least one of which mediates signal transduction in response to GDNF by activating the cytoplasmic tyrosine kinase Fyn (3). In addition to its activities on ventral midbrain dopaminergic neurons, GDNF has been shown to promote survival, neurite outgrowth and guidance in several subpopulations of central and

peripheral neurons (4, 5). In agreement with those observations, knock-out mice lacking GDNF or GFR β 1 display various deficits among subpopulations of sensory and enteric neurons (6). However, no abnormalities during brain development have so far been uncovered in those mice, which die prematurely a few hours after birth due to kidney agenesis and lack of enteric neurons (6). We set out to investigate possible functions of GDNF signaling during brain development *in vivo* by first studying the patterns of expression of GDNF and its receptors in the embryonic and early postnatal forebrain. We found that GDNF and GFR β 1 were expressed in the two main pathways of tangential cell migration in the brain: the rostral migratory stream (RMS) and along the pathway connecting the ganglionic eminences with the developing cerebral cortex. These observations prompted us to investigate possible roles for GDNF signaling in the differentiation and migration of GABAergic precursors in the

olfactory bulb and cerebral cortex. Our results indicate that different receptor complexes mediate the effects of GDNF family ligands on tangential cell migration in the developing nervous system.

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