A Novel VIP Responsive Gene

Activity Dependent Neuroprotective Protein

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Abstract: Activity dependent neuroprotective protein (ADNP, 828 amino acids, pI 5.99) is a glial-derived protein that contains a femtomolar active neuroprotective peptide, NAPVSIPQ (NAP). VIP induces a two- to threefold increase in ADNP mRNA in astrocytes, suggesting that ADNP is a VIP-responsive gene. ADNP is widely distributed in the mouse hippocampus, cerebellum, and cerebral cortex. VIP has been shown to possess neuroprotective activity that may be exerted through the activation of glial proteins. We suggest that ADNP may be part of the VIP protection pathway through the femtomolar-acting NAP and through putative interaction with other macromolecules.

The Discovery of ADNP

VIP was originally shown to protect spinal cord neurons against death associated with electrical blockade. However, for VIP to exert neuroprotective activity in this experimental paradigm, glial cells possessing VIP receptors were essential. VIP-associated neuroprotection was then found to require, in part, the PAC1 receptor (splice variant hop2) and a cGMP-generating system. Furthermore, the amino acids in VIP (shared also by PACAP) and attached to a steric acid moiety, stearyl-KKYLNH2 (VIP20–23), mimicked the VIP neuroprotective activity. Studies have now been initiated in search for the mediators of VIP-associated neuroprotection. These experiments led to finding interleukin 1 alpha, protease nexin 1, activity-dependent neurotrophic factor, and heat shock protein 60 as proteins acting downstream of VIP.

Activity dependent neurotrophic protein was found to share a sequence with hsp60; that is, VLGGGSALLRSIPA (ADNF-14). ADNF-14 that has serines instead of the cysteines found in hsp60, exhibited increased immunogenicity and most importantly, it exhibited neuroprotective activity by itself. Structure–activity experiments identified SALLRSIPA (ADNF-9) as the active core of ADNF, a femtomolar acting nine-aminoacid peptide.

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Antibodies to SALLRSIPA identified NAPVISPQ (NAP), a peptide moiety in the newly cloned protein, activity-dependent neuroprotective protein, ADNP. These antibodies were in fact used in screening a cDNA expression library that originated in a mixed neuronal–glial cell population. SALLRSIPA exhibited as yet unprecedented neuroprotective activity in vitro, protecting neurons against death associated, not only with electrical blockade, but also with the beta amyloid peptide (the Alzheimer related neurotoxin), gp120 (the toxic envelope protein of the human immunodeficiency virus), and excitotoxicity (N-methyl-D-aspartate). NAPVISPQ exhibited a similar neuroprotective profile to SALLRSIPA in vitro. However, in vivo, in an apolipoprotein-E deficient mouse model, daily injection of NAPVISPQ, but not of SALLRSIPA resulted in increased cholinergic activity and enhanced learning capacities a week after cessation of treatment. Thus, it is of further interest to understand the biology of the NAPVSIPQ precursor, ADNP.

Incubation of rat cerebral astrocytes with nanomolar concentrations of VIP induced a two- to threefold increase in ADNP mRNA in astrocytes, suggesting that ADNP is a VIP responsive gene. Furthermore, ADNP mRNA was found to be widely distributed in the mouse hippocampus, cerebellum, and cerebral cortex, suggesting a brain function.

**SELECTED MOTIFS IN ADNP**

As previously reported, the sequence of ADNP contained (1) a neuroprotective peptide, NAPVSIPQ, sharing structural and immunological homologies with the previously reported, ADNF (see above); (2) a glutaredoxin active site; (3) a zinc binding domain; and (4) a putative signal peptide that may indicate a secreted protein. Previously, proteins that do not possess a classical signal peptide were shown to be secreted. Examples include, the TAT transcription factor of the human immunodeficiency virus, fibroblast growth factors, lactoferrin, and the engrailed homeoprotein. Furthermore, our studies suggested the secretion of hsp60. A unifying theme for these proteins is their nuclear localization, or their mitochondrial localization (hsp60). Leucine-rich nuclear export signals have been identified and leucine-rich sequences found in protein sequences not associated with the N-terminal of the protein molecule were suggested to be involved in protein secretion. Our results indicate that ADNP contains a leucine-rich sequence (see FIGURE 1), and leucine-rich sequences are also observed in hsp60 (e.g., VLGGCALLRCIPALASL) and in ADNF (e.g., VLGGGSALLRSIPALASL). The identification of a leucine-rich motif in ADNP

![FIGURE 1. The leucine-rich nuclear export sequence of chicken engrailed protein (CHICK EN2, Refs. 18, 19) is compared to a similar sequence found in ADNP, not only are the leucines and isoleucines (bold) similarly distributed but there are other identical amino acids (italics and underlined).](image)
suggests an alternative route of export. This, coupled with the many putative proteolytic signals in ADNP, may in fact suggest ADNP as a source for bioactive peptides, such as NAPVSIPQ.

CONCLUSIONS AND WORKING HYPOTHESIS

When VIP binds to the target glial cells changes occur, and multiple proteins are released. The studies described above identify just a fraction of the proteins associated with VIP activity in the nervous system. It is clear that proteins with different functions and representing different families are involved, including proteins associated with the immune system (i.e., interleukins), proteins associated with protein degradation (i.e., protease nexin I), proteins associated with stress responses (i.e., hsp60), and novel protein sequences (i.e., ADNF and ADNP). These proteins may interact with each other, indeed ADNF-9 (the active peptide of ADNF) induces increases in hsp60 (mRNA and protein) in neurons. Future studies should determine the pharmacological, temporal, cellular, and subcellular interactions of the VIP-responsive proteins that may offer better understanding of neuronal/glial functionality.

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REFERENCES


