A Femtomolar Acting Octapeptide Interacts with Tubulin and Protects Astrocytes against Zinc Intoxication*

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An octapeptide was previously described that protects neurons against a wide variety of insults directly and indirectly as a result of interactions (at femtomolar concentrations) with supporting glial cells. The current study set out to identify the octapeptide binding molecules so as to understand the high affinity mechanisms of cellular protection. Studies utilizing affinity chromatography of brain extracts identified tubulin, the brain major protein, as the octapeptide-binding ligand. Dot blot analysis with pure tubulin and the biotinylated octapeptide verified this finding. When added to cerebral cortical astrocytes, the octapeptide (10^{-12}-10^{-10} \text{ M}) induced a rapid microtubule reorganization into distinct microtubular structures that were stained by monoclonal tubulin antibodies and visualized by confocal microscopy. Immunoblot analysis of brain extracts revealed that the octapeptide induced a similar change and was detected in the intracellular milieu, even when cells were incubated at 4 °C or at low pH. In a cell-free system, the octapeptide stimulated tubulin assembly into microtubules. Furthermore, treatment of astrocytes with zinc chloride resulted in microtubule disassembly and cell death that was protected by the octapeptide. In conclusion, the results suggest that the octapeptide crosses the plasma membrane and interacts directly with tubulin, the microtubule subunit, to induce microtubule reorganization and improved survival. Because microtubules are the key component of the neuronal and glial cytoskeleton that regulates cell division, differentiation, and protection, this finding may explain the breadth and efficiency of the cellular protective capacities of the octapeptide.

The octapeptide NAP,1 Asn-Ala-Pro-Val-Ser-Ile-Pro-Gln (single-letter code: NAPVSIPQ), is a femtomolar-acting neuroprotective peptide derived from activity-dependent neuroprotective protein (ADNP) (1,2). The discovery of NAP resulted from studies on the major neuroprotective, vasoactive intestinal peptide (VIP). VIP, released from nerve cells, provides neuronal protection by inducing the synthesis and secretion of neuroprotective proteins from astrocytes (3,4). ADNP was identified as a VIP-responsive glial protein (1,5), and VIP neuroprotection was suggested to be mediated in part through increased synthesis of ADNP (5). Decreased ADNP synthesis by antisense oligodeoxynucleotides resulted in increased p53 expression and cell death (2).

NAP, an eight-amino acid peptide derived from ADNP that was identified by peptide activity scanning, was shown to be neuroprotective in a wide variety of systems and against multiple neurotoxins (6). NAP provided neuroprotection at remarkably low concentrations against the Alzheimer’s disease toxin (β-amyloid peptide) (1,7), the toxic envelope protein of the human immunodeficiency virus (HIV; gp120) (1), glucose deprivation (7), electrical blockade (tetrodotoxin) (1), oxidative stress (hydrogen peroxide) (8), dopamine toxicity, and decreased glutathione (9), in vitro. Further studies indicated an exceptionally broad range of neuroprotective concentrations against excitotoxicity (N-methyl-D-aspartate; 10^{-16}-10^{-8} \text{ M}) (1). Treatment of mixed neuralglial populations identified two peaks of activity at femtomolar and at nanomolar concentrations; in contrast, treatment of enriched neuronal populations indicated only one peak of activity, at nanomolar concentrations of NAP. These results suggest a direct and an indirect mode of action for NAP, with the latter involving glial cells (1,7,10).

In vivo, NAP was identified as a potent neuroprotective peptide in a mouse model of apolipoprotein E deficiency (knockout) (1) and in a rat model of cholinotoxicity (11). The lipid carrier, apolipoprotein E, has been implicated as a risk factor in Alzheimer’s disease, and because the knock-out mice exhibit short-term memory deficits that are ameliorated by chronic peptide treatment, NAP holds promise for future treatment against disease-related short-term memory deficits. Furthermore, Alzheimer’s disease is associated with death of cholinergic neurons. Rats treated with the cholinotoxin ethylcholine, aziridinium, provide a model of cholinotoxicity. A week following a single intracerebroventricular injection of the cholinotoxin, NAP administration ensued using daily intranasal application. Significant improvements in short-term spatial memory in NAP-treated animals were observed (11). Cognition enhancement was also found in the Morris water maze paradigm in middle-aged rats treated daily (by intranasal administration) with NAP (12).

In acute in vivo models of neuronal damage, a single NAP subcutaneous injection after closed head injury dramatically reduced mortality and facilitated clinical recovery (motor ability, balancing, and alertness) in mice (13). Water accumulation (edema) was reduced by 70% in the NAP-treated mice. Magnetic resonance imaging demonstrated significant brain tissue...
recovery in NAP-treated animals. Pretreatment with NAP by daily subcutaneous injections for the first 3 months of life followed by head trauma at the age of 4 months resulted in faster recovery and enhanced performance in a learning and memory paradigm (14). Middle cerebral artery occlusion in rats is manifested in stroke-like symptoms, including infarct formation, neuronal cell death, motor disabilities, and sensory impairments. A single intravenous NAP injection up to 4 h following artery occlusion resulted in a significant reduction in infarct size, marked protection against neuronal death (apoptosis), and defense against motor and sensory disabilities (15). Both closed head injury and middle cerebral artery occlusion can be considered as acute brain damage with severe secondary outcome that could be prevented, in part, by neuroprotection. In both cases, oxidative stress that is a result of the initial insult may exacerbate the damage. In this respect, fetal demise and growth restriction caused by overexposure of pregnant mice to alcohol are thought to be because of severe oxidative damage. In pregnant mice exposed to one intraperitoneal injection of alcohol, fetal demise was inhibited by a single injection of NAP (16).

Given the breadth of the protective activities of NAP, its mechanism of action is of great interest. Potential signal transduction pathways include cGMP production (17) and interference with inflammatory mechanisms, tumor necrosis factor α, and MAC1-related changes (13, 14, 18). NAP-associated protection against oxidative stress (8, 9), glucose deprivation (7), and apoptotic mechanisms (15) suggests interference with fundamental processes. The current study identifies the tubulin subunit of microtubules as the primary target of NAP, disclosing that indeed NAP interacts with a key component of the cell and facilitates survival.

EXPERIMENTAL PROCEDURES

Affi-Gel 10 NAP Affinity Chromatography—A protein lysate was prepared from 1-day-old rat brains in a buffer containing the following ingredients: 150 mM NaCl, 1 mM EDTA, 50 mM Tris–HCl, pH 4.5, 0.1% Triton X-100, 1% Nonidet P-40, and a protease inhibitor mixture (Roche Diagnostics). DNA was fragmented by sonication. Cell debris was discarded following 30 min of centrifugation at 30,000 × g. An affinity column containing NAP was prepared using elongated NAP (KKKGGNAPVSPQG, synthesized as before) (1, 11) and Affi-Gel 10 in 0.2 M NaHCO₃, 0.5 mM NaCl, pH 7.5. Further column preparation was according to the manufacturer’s instructions (Amersham Biosciences). The brain extract prepared as above was loaded (2 mg/ml) on the column at 20 °C and incubated for an hour; the column was then washed with phosphate-buffered saline until all unbound protein eluted (as confirmed by protein assay (Bradford; BioRad). NAP-binding proteins were eluted in 0.1 M glycine, pH 3.0, from an Affi-Gel 10-NAP affinity column; lane 5, NAP-sulfolink wash through protein; lane 6, NAP-binding proteins displaced (eluted) from the NAP-sulfolink affinity column by competition with excess soluble NAP. (MW, the molecular mass in kDa).

Sulfolink Coupling Gel NAP Affinity Chromatography—The second isolation efforts utilized a different affinity column, sulfolink coupling gel (Pierce). Binding of CKKGGGNAPVSPQG was performed according

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**Fig. 1.** Affinity chromatography: isolation of NAP-binding proteins. NAP was bound to either Affi-Gel 10 or sulfolink coupling gel, and protein extracts from 1-day-old rat brains were loaded on the resulting affinity columns (see “Experimental Procedures”). Proteins from the different stages of the purification procedure were separated on a 12% polyacrylamide SDS-containing gel followed by GelCode Blue protein stain (Pierce). The stained gel lanes depicted on the figure contain the following protein samples: lanes 1 and 4, protein molecular mass markers (BioRad); lane 2, initial brain extract; lane 3, NAP-binding proteins that were eluted with 0.1 M glycine, pH 3.0, from an Affi-Gel 10-NAP affinity column; lane 5, NAP-sulfolink wash through proteins; lane 6, NAP-binding proteins displaced (eluted) from the NAP-sulfolink affinity column by competition with excess soluble NAP. (MW, the molecular mass in kDa).

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**Fig. 2.** Identification of tubulin as a NAP-binding protein. A, sequence analysis. The major NAP-binding protein band observed in Fig. 1, lane 3 (−50,000 daltons) was excised, and subjected to in-gel proteolysis with trypsin and mass spectrometry. Results identified the sequence depicted on the figure. B, dot blot analysis of biotin-labeled NAP and tubulin. Purified tubulin (Sigma) was bound to a nitrocellulose membrane. The membrane was then incubated in a blocking solution, followed by a second incubation with biotin-labeled NAP and detection by avidin-horseradish peroxidase conjugate and ECL+. For further details, see “Experimental Procedures.”

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**Table:**

| Accession | g223556 |
|-----------|---------|
| Version   | GI:213356 |

A

DEFINITION tubulin alpha (gene bank).

### DEFINITION

**tubulin alpha (gene bank).**

**Accession:** g223556

**Version:** GI:213356

1 mrecsihevgqsgvqignac welyclebqql pqpqgmpqdk tigpdpdefa tffpsetsgdk
61 tppravydrl eptntvedrt gtyqrlilpse qltsgedaa mynyguytyt gkeidirdvld
121 mpkldadqg tfgqflvfhf fggggtqegf slerlsdvd ygksklefs lypapqvsta
181 vvepynilsit thtthheshd afmvdmealy diocrnlude rpyttmlnel lqojvesita
241 elrfsga iniq diteqfnvl pyprhflad tpyvseseqk iyheqleaae itnactepan
301 qmvkqdpcoh kymaccllyz gdvqykgvaa dsiatktktq ifqvdcwctp fgvyginyopp
361 trypggdlak vgavrulnsn ttiaeseawac lohdflmls krafvhwvgy egmeegefse
421 arehndalek dveeyvqvdv eyseeeegge y

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**B**

Tubulin

(bovine brain)
to the manufacturer’s instructions. Brain extract was prepared as above. Binding was performed at 4 °C for 20 h, washing was as above, and elution was performed with NAP (NAPVSIPQ) in 2 mg/ml phosphate-buffered saline (2 ml/2-ml column) at 4 °C for 20 h.

**Sequence Analysis**—To further identify the NAP-binding protein, the polyacrylamide gel portion containing the major purified protein band was subjected to in-gel proteolysis with trypsin and mass spectrometry analysis by the Smoler Protein Center, Department of Biology, The Technion, Israel Institute of Technology.

**Direct NAP Binding to Protein, Dot Blot Analysis**—The protein (1–4 μg/1 μl) was applied to nitrocellulose membrane (Schleicher & Schuell, Dassel, Germany), 1 μl/spot, and dried (45 min, 20 °C). The membrane was incubated in a blocking solution (10 mM Tris, 6 mM NaCl, 0.05% Tween 20, and 10% low-fat milk) for 16 h at 4 °C. Detection was with biotin-labeled NAP (w/w, NAP/tubulin; Sigma) (19), avidin-horseradish peroxidase conjugate, and ECL+ (Western blotting detection system; Amersham Biosciences).

**Cell Cultures**—Rat cerebral cortical cells from newborn pups were prepared as before (1). In short, cerebral cortical tissue was incubated for 20 min at 37 °C in Hank’s balanced salt solution containing 15 mM HEPES, pH 7.3 (Biological Industries, Beit Haemek, Israel), and trypsin. Dissociated cerebral cortical cells were added to the culture dish with 5% horse serum in Dulbecco’s modified Eagle’s medium. Cells were plated in a ratio of 1 cortex/two 75-cm² cell culture flasks (polystyrene; Corning Glass). The medium was changed 1 day after plating. Cells were split after 10 days of incubation, plated in 24-well plates (each flask into 60 wells containing 250 μl of medium), and incubated for an additional 2 weeks. In some experiments, ZnCl₂ (150 μM; Sigma) (20) was added for an additional incubation of 16 h. NAP (10⁻¹⁵–10⁻¹⁰ M) was added either together with ZnCl₂ or 3 h after the addition of the ZnCl₂ solution.

![Confocal microscopy, NAP, and tubulin in rat cerebral cortical astrocytes. A, confocal microscopy. NAP (10⁻¹⁵ or 10⁻¹⁵ M) was added to 2-week-old astrocyte cultures and incubated for various times (0, no peptide addition; 2, 2 h; 4, 4 h; 24, 24 h). Control peptide (C2 = VLGGSALL, 10⁻¹⁵ M) was incubated for the same time periods. Tubulin was detected with mouse monoclonal antibodies (TUB2.5) (21) and secondary rhodamine-labeled goat anti-mouse IgG. (Bars, 20 μm). B, quantitation of cells displaying organized microtubular structure and cells displaying disorganized microtubular structures. 150–225 cells stained with antitubulin antibodies (as in panel A) were analyzed for each time point of incubation with NAP. Incubation periods were 0, 2, 4, and 24 h.](image-url)
Metabolic Activity Measurements—Metabolic activity of viable cells in culture was measured by a calorimetric method using a tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, MTS) and an electron-coupling reagent, phenazine methosulfate. MTS is bioreduced by the living cells to the formazan form that is detected at 490 nm (Promega, Madison, WI).

Confocal Microscopy—Synthetic NAP (1) or fluorescein-labeled NAP (19) was added to tissue culture cells in ascending concentrations and incubated for 2 h at 37 °C. Fig. 3 shows the visualization by confocal microscopy. Two-week-old astrocyte cultures were incubated with fluorescein-labeled NAP, and tubulin was detected with mouse monoclonal antibodies (TUB2.5) (21) and secondary rhodamine-labeled goat anti-mouse IgG. A, the astrocyte cultures were incubated with NAP (10^{-10} M) for 2 h at 37 °C. B, varying incubation periods and conditions were implemented in the cultures, (pH 3), (15 min) and 4 °C (cold, 1 h) with 10^{-15} M NAP and 4 °C (cold, 30 min) with 10^{-10} M NAP as depicted on the figure. NAP is depicted with green dots, fluorescein, and tubulin in red microtubule structures, rhodamine. Bars, 10 μm.

Fig. 4. Double labeling with fluorescein-NAP and monoclonal tubulin antibodies: visualization by confocal microscopy. Two-week-old astrocyte cultures (as in Fig. 3) were incubated with fluorescein-labeled NAP, and tubulin was detected with mouse monoclonal antibodies (TUB2.5) (21) and secondary rhodamine-labeled goat anti-mouse IgG. A, the astrocyte cultures were incubated with NAP (10^{-10} M) for 2 h at 37 °C. B, varying incubation periods and conditions were implemented in the cultures, (pH 3), (15 min) and 4 °C (cold, 1 h) with 10^{-15} M NAP and 4 °C (cold, 30 min) with 10^{-10} M NAP as depicted on the figure. NAP is depicted with green dots, fluorescein, and tubulin in red microtubule structures, rhodamine. Bars, 10 μm.

Fig. 5. NAP promotes tubulin assembly. Tubulin assembly was performed as described under “Experimental Procedures.” Paclitaxel served as a positive control for enhanced tubulin assembly, and C2 = VLGGGSALL was a negative control for NAP (see above). NAP (nap) concentration was 10^{-15} M (–15). Tubulin alone, squares; NAP, open circles; paclitaxel, triangles; C2, closed circles. The results are means ± S.E. of two or three independent experiments performed in triplicate. Statistical analysis of time-response data, including 0, 10, 20, 30, and 40 min after the treatment, performed by analysis of variance followed by Dunnett’s multiple comparison test indicated that in the presence of 10^{-15} M NAP, significant increases were observed with p < 0.05 as the minimum significance level. Similar significance levels were observed for paclitaxel-enhanced tubulin assembly.
incubated for 15 min to 24 h. After incubation, cells were extensively washed and fixed in 4% paraformaldehyde. After fixation, Triton X-100 was added to allow antibody cellular penetration using mouse monoclonal tubulin antibodies (TUB2.5) (21) and rhodamine-labeled secondary goat antimouse IgG (Jackson ImmunoResearch, West Grove, PA). Fluorescently stained cells were analyzed using a Zeiss confocal laser scanning microscope. The Zeiss LSM 410 inverted (Oberkoch, Germany) is equipped with a 25-milliwatt krypton-argon laser (488 and 568 maximum lines) and 10-milliwatt helium-neon laser (633 maximum lines). A ×40 numeric aperture/1.2 C Apochromat water-immersion lens (Axiovert 135 M; Zeiss) was used for all imaging.

Microtubule Assembly—CytoDYNAMIX Screen 01 (CDS01), a microtubule assembly kit, was obtained from Cytoskeleton (Denver, CO) (www.cytoskeleton.com). Bovine microtubule-associated protein-rich tubulin (HTS01) was resuspended in G-PEM buffer (80 mM PIPES, pH 6.9, 1 mM MgCl₂, 1 mM EGTA, and 1 mM GTP) and subjected to polymerization at 37 °C. The reaction was performed in a 96-well plate. Assembly was monitored with a spectrophotometer, SPECTRAMax 190 (Molecular Devices, Sunnyvale, CA), employing continuous recordings at 350 nm.

Statistical Analysis—All values are given as means ± S.E. from a number of independent experiments as indicated in the figure legends. Results were analyzed for statistical significance by Student’s t test and analysis of variance followed by Dunnet’s multiple comparisons test.

RESULTS

Isolation of NAP-binding Proteins by Affinity Chromatography—Brain homogenates were chosen as a putative enriched source for NAP-interacting molecules. Extracts were subjected to affinity chromatography comprising NAP bound to two different solid supports, Affi-Gel 10 and sulfolink coupling gel. Elution of the NAP-interacting molecules was obtained by either reducing the pH or by competing the binding to the insoluble NAP with excess free soluble NAP. Electrophoresis on a 12% polyacrylamide SDS-containing gel revealed a major purified protein band at about 50,000 daltons (Fig. 1); this protein was subjected to further characterization.

Tubulin Is a NAP-binding Protein—When the gel portion containing the purified −50,000-dalton band protein was submitted to in-gel proteolysis with trypsin and mass spectrometry analysis, this NAP-binding protein was identified as rat-a tubulin (molecular mass 50,242), gi223556 (Fig. 2A). The identification of tubulin included the characterization of 6 different tryptic peptides (Fig. 2A, bold). A dot blot assay performed with spotted tubulin indicated NAP binding to tubulin (Fig. 2B).

NAP Interaction with Tubulin/Microtubules: Confocal Microscopy—To establish an association between tubulin (22, 23) and NAP in the living cell, we initiated confocal microscopy analysis of fluorescent NAP and immunodetection of tubulin (21). To visualize the microtubule structure, monoclonal β-tubulin antibodies (TUB2.5) (21) were used to stain primary astrocyte cultures. Either fluorescein-labeled NAP or native NAP was added to 2-week-old astrocyte cultures. Astrocytes were used as a model because previous results have indicated that, although nanomolar concentrations of NAP protected neuronal enriched cultures against β-amyloid toxicity (7), a more potent protection at femtomolar concentrations of NAP was observed when neurons were plated on a bed of astrocytes (1). A time course experiment suggested that the microtubule reorganization was apparent in astrocytes 2 h after NAP application, with an additional condensation 4 h after application, and returned to the original morphology 24 h after NAP application (Fig. 3A). Mitotic spindles were not noticeable. Similar microtubule reorganizations were observed with NAP at concentrations ranging from 10⁻¹⁵ to 10⁻¹⁰ M with fluorescein-labeled and with native NAP. Evaluation of the number of cells undergoing microtubule reorganization following NAP treatment showed maximal organization at 2–4 h with a decline at 24 h (Fig. 3B). A control peptide, C2 (VLGGGSALL), that does not protect cells in vitro (24) did not induce a microtubule-associated morphological change (Fig. 3A).

Detection of Fluorescein-labeled NAP—After a 2-h incubation at 37 °C, fluorescein-labeled NAP was detected inside the cell (Fig. 4A). A critical question is whether NAP induces microtubule reorganization through interaction with a surface receptor or whether it is a pore-forming peptide that interacts with the
lipid bilayer and is then internalized into cells. To evaluate potential surface labeling, the initial incubation was carried out at 4 °C and in a parallel experiment at pH 3.0. When NAP (10⁻¹⁰ M) was incubated with astrocytes at pH 3.0 for 15 min (Fig. 4B), microtubule reorganization was apparent and fluorescein-labeled NAP was visualized inside the cells. At 4 °C, although microtubule reorganization did not take place because microtubules undergo disassembly at 4 °C (25), a dose-dependent intracellular accumulation of NAP was apparent (Fig. 4B).

**NAP Promotes Tubulin Assembly**—Using a high through-put analysis kit containing bovine tubulin (Cytoskeleton), tubulin assembly was determined in the presence of increasing NAP concentrations. Measurements included absorbance determinations at 350 nm. Although 10⁻¹⁰ M NAP did not influence microtubule assembly in the test tube (data not shown), 10⁻¹⁰ M NAP stimulated microtubule assembly in a similar way to paclitaxel (Fig. 5). Paclitaxel, a known tubulin stabilizing agent also suggested as a neuroprotective agent (26), was used as a positive control. NAP at 10⁻¹⁰ M promoted tubulin assembly to the same degree as at a concentration of 10⁻¹⁰ M (data not shown). C2, a peptide that was utilized as a negative control in the cellular assay (Fig. 3A), did not affect microtubule assembly as well.

**NAP Protects against Zinc Intoxication**—When 2-week-old astrocyte cultures were exposed to ZnCl₂ (16 h of incubation), there was an 88 ± 0.008% reduction in cellular metabolic activity (Fig. 6). This marked reduction in metabolic activity (associated with cellular viability) was coupled to changes in microtubule organization observed 4 h after the addition of ZnCl₂ (Fig. 7). NAP added together with ZnCl₂ at concentrations ranging from 10⁻¹⁰ to 10⁻⁰ M protected completely against ZnCl₂ intoxication (Fig. 6, p < 0.01). Furthermore, the cellular microtubule network assumed the same organization when NAP was added in the presence or in the absence of ZnCl₂ (Fig. 7). Cell counts 4 h after the addition of NAP or ZnCl₂, or both, showed that of 100 astrocytes treated with ZnCl₂, 73 showed condensed tubulin structures as depicted in Fig. 7. In contrast, all the NAP-treated cells showed organized microtubular structures, and of 101 cells treated with ZnCl₂ and NAP, 72 cells showed organized microtubular structures as depicted in Fig. 7.

**DISCUSSION**

The present report outlines a mechanism of action for the short protective peptide, NAP. Affinity chromatography coupled to mass spectrometry identified tubulin, the subunit protein of microtubules, as a major NAP-binding protein. NAP internalization into astrocytes was observed at low pH and low temperatures, suggesting cellular penetration without a classical peptide receptor. Incubation of astrocytes with NAP resulted in time-dependent microtubule reorganization. Furthermore, NAP accelerated microtubule assembly in vitro. Finally, NAP protected astrocytes against ZnCl₂ toxicity, a protection that was paralleled with microtubule reorganization.

Generally speaking, cellular membranes are composed of lipid-protein bilayers that are freely permeable to small, non-ionic lipophilic compounds and are inherently impermeable to polar compounds, macromolecules, and therapeutic or diagnos-

### Table I

NAP exhibits similarities to membrane translocation polypeptides

| Translocation Peptide                      | Homology to NAP                  | % similarity | Reference and Accession number |
|-------------------------------------------|----------------------------------|--------------|--------------------------------|
| VP22 translocation domain from HSV (aa 19-36) | NAPVSIPQ | 100.0% identity in 4 aa overlap | 11S71935 |
| pertussis toxin subunit S1 precursor (21-34) | NAPVSIPQ | 66.7% identity in 6 aa overlap | 11S25973 |
| Kaposi fibroblast growth factor (aa 29-40) | NAPVSIPQ | 50.0% identity in 4 aa overlap | 11S291998 |
| adenylate cyclase toxin activator (aa 45-56) | NAPVSIPQ | 42.9% identity in 7 aa overlap | 11Q45599 |
| diphtheria toxin (aa 424-435) | NAPVSIPQ | 50.0% identity in 6 aa overlap | 11P00588 |
| pertussis toxin subunit S2 precursor (aa 28-39) | NAPVSIPQ | 50.0% identity in 4 aa overlap | 11P04978 |
| pertussis toxin subunit S3 precursor (aa 28-40) | NAPVSIPQ | 57.1% identity in 7 aa overlap | 11P04979 |
| pertussis toxin subunit S5 precursor (aa 14-25) | NAPVSIPQ | 20.0% identity in 5 aa overlap | 11P04981 |
Octetopeptide and Tubulin

In conclusion, NAP, which provides protection at femtomolar concentrations, seems to interact with the glial tubulin cytoskeleton after cellular internalization, a process which does not seem to require a conventional peptide-receptor interaction. The NAP doses required for tubulin polymerization concerned with the doses required for cellular protection against ZnCl₂ intoxication that was associated with microtubule reorganization. NAP mimicked the effects observed with paclitaxel, which is also suggested as a cytotoxic agent (26, 51). Furthermore, neurosteroids (pregnenolone) are suggested to offer cytoprotection through interaction with the neural microtubule-associated protein type 2 and to increase both the role and extent of tubulin polymerization (44). Because the tubulin cytoskeleton is a key component of the maintenance and stability of the nervous tissue, the results presented above explain in part the breadth and potency of the protective effect of NAP.

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