Neuronal Pentraxin Receptor, a Novel Putative Integral Membrane Pentraxin That Interacts with Neuronal Pentraxin 1 and 2 and Taipoxin-associated Calcium-binding Protein 49*

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We have identified the first putative integral membrane pentraxin and named it neuronal pentraxin receptor (NPR). NPR is enriched by affinity chromatography on columns of a snake venom toxin, taipoxin, and columns of the taipoxin-binding proteins neuronal pentraxin 1 (NP1), neuronal pentraxin 2 (NP2), and taipoxin-associated calcium-binding protein 49 (TCBP49). The predominant form of NPR contains an putative NH2-terminal transmembrane domain and all forms of NPR are glycosylated. NP1 has 49 and 48% amino acid identity to NP1 and NP2, respectively, and NPR message is expressed in neuronal regions that express NP1 and NP2. We suggest that NPR, NP1, NP2, and TCBP49 are involved in a pathway responsible for the transport of taipoxin into synapses and that this may represent a novel neuronal uptake pathway involved in the clearance of synaptic debris.

We identified two taipoxin binding proteins for a presynaptic-acting snake venom neurotoxin, taipoxin, that blocks recycling of synaptic vesicles (1, 2). Affinity chromatography of solubilized rat brain membranes on columns of immobilized taipoxin enriches two major proteins: (i) neuronal pentraxin 1 (NP1),1 a neurally secreted protein with homology to serum pentraxins (2), and (ii) taipoxin-associated calcium-binding protein 49 (TCBP49), a reticular calcium-binding protein (3). NP1 has homology to previously identified pentraxins, such as serum amyloid P protein and C-reactive protein, which are elevated in the serum during acute phase response. Although the exact functions of these previously identified pentraxins are not known, they have been shown to bind, in a calcium-dependent manner, a wide variety of ligands and have been proposed to mediate the uptake of bacteria, toxins, and extracellular debris (4, 5). Homology to serum pentraxins, as well as the presence of a cleaved signal peptide and N-linked glycosylation sites, suggests that NP1 is secreted. The abundance of NP1 mRNA and rarity of NP1 protein suggest that NP1 protein has a rapid turnover. We have proposed that NP1 has a role in uptake at the synapse and that NP1 mediates the uptake of taipoxin into neurons. By low stringency screening, we identified an additional neuronal pentraxin (NP2) in human that has 54% amino acid identity with NP1 and is expressed in brain and multiple other tissues (6). Potential homologs of NP2 have been identified in guinea pig as a sperm acrosomal protein, apoxin/p50 (7, 8), and in rat as a neural activity-regulated pentraxin, narp (9). The second taipoxin-binding protein, TCBP49, binds calcium via six EF-hand calcium binding motifs and is localized to the lumen of reticular membranes in neurons and glia (3). It contains the carboxy-terminal sequence HDEL which has been shown to occasionally mediate endoplasmic reticulum retention in mammalian cells (10–12). We have suggested that NP1 binds to synaptic material and is taken up into a compartment containing TCBP49 (2, 3). We have also suggested that NP1 allows the internalization of taipoxin or a taipoxin/NP1 complex and that this uptake plays a role in the molecular mechanism of taipoxin toxicity.

To identify other proteins in such an uptake pathway, we have chromatographed rat brain proteins on columns of immobilized taipoxin, recombinant TCBP49, and glutathione S-transferase-NP1/NP2 fusion proteins. Here we report the identification, cloning, and cDNA sequence of a protein, neuronal pentraxin receptor (NPR), that binds tightly to taipoxin, TCBP49, NP1, and NP2 columns.

**Materials—** Taipoxin was purified from Oxyuranus scutellatus venom by gel exclusion chromatography as described (13).32P-Labeled nucleotides were obtained from NEN Life Science Products. Restriction and DNA modification enzymes were from New England Biolabs and Promega. Sequenase was from U. S. Biochemical Corp. Protein molecular weight standards were obtained from Bio-Rad and RNA and DNA standards from Life Technologies, Inc. Peroxidase-labeled secondary antibodies were from Cappel. All other chemicals and proteins were of reagent grade and used without further purification.

**Bacterial Expression and Purification of TCBP49, GST-NP1, and GST-NP2—** A piece of TCBP49 cDNA coding for the entire TCBP49 protein minus its NH2-terminal signal sequence was amplified by PCR using a 5’ oligonucleotide (GGTGGCCATGGGCCAGCAAGCGAG-GAGCTG) that creates an NcoI site and initiator methionine codon at nucleotide 293 of the cDNA, just 3’ to the sequence coding for the signal peptide, and a 3’ oligonucleotide (CATAAAATGTATAGCCTAGG-CAAACTCAGAAGGC) to create a BglII site 3’ to the stop codon. After restriction digest, the NcoI-BglII PCR fragment was subcloned between the NcoI and BamHI sites of the bacterial expression vector pET 11d (Novagen, Inc., Madison, WI). Bacteria containing this vector were induced with isopropyl-β-D-thiogalactopyranoside (14), pelleted, resuspended in buffer (below), and lysed by French press. rTCBP49 was purified by taipoxin chromatography. Taipoxin was coupled to activated CH-Sepharose 4B (Pharmacia Biotech Inc.) according to the commercial protocol. The column was equilibrated in 20 mM Hepes, pH 7.4, 100 mM NaCl, and 1 mM CaCl2, and rTCBP49-containing bacterial supernatant was applied to the column in the same buffer with protease inhibitors. Flow-through was collected and saved. The column was washed with...
equilibration buffer. rTCBP49 was eluted with the same buffer containing 10 mM EDTA and concentrated with a centrprep 30 (Amicon). This procedure yields rTCP49 that is 95–98% pure as judged by Coomassie staining. The piece of rat NP1 cDNA coding for the entire NP1 protein minus its NH2-terminal signal sequence was amplified by PCR using a 5' oligonucleotide (TCTTGGAAATCCGGCCAGAGGTCGG) that creates an EcoRI site just 3' to the region encoding the signal sequence and a 3' oligonucleotide (GAAGGAGAGATGGTGACGACACAGGAGGGCAGG) that creates a SaI site 3' to the stop codon. After restriction digest, the EcoRI-SaI PCR fragment was subcloned into the bacterial expression vector pGEX KG (15), positioning the cDNA downstream, and in frame with the glutathione S-transferase gene. To create the GST-NP2 expression construct, a similar portion of NP2 coding sequence was amplified by PCR using 5' and 3' oligonucleotides (GCCGGGATCCTGAATGAGGTGGCCCCTCATGCCTTAGC and CAAGGGAGTCTCAGGAAGTGGGTCCTGAGCTCATTAG) to create BamHI and EcoRI sites, respectively, for insertion into the expression vector pGEX KT (16). For protein expression, bacteria containing the pGEX KG-NP1 or pGEX KT-NP2 plasmids were induced with isopropyl-β-D-thiogalactopyranoside. The bacteria was pelleted and lysed by French press in 20 mM Heps, pH 7.4, 10 mM EDTA containing 0.25 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin and pepstatin. Insoluble material containing most of the GST-NP1 and GST-NP2 proteins was pelleted, washed in 100 mM NaCl, 0.2% Triton X-100, 1 mM CaCl2, then eluted with 150,000 g supernatant was dialyzed at 4°C against 10 mM Heps, pH 7.4, 5 mM EDTA, and finally resuspended in 0.25 M NaCl, 0.2% Triton X-100, 1 mM EGTA. Insoluble material was removed by centrifugation, and the supernatant was dialyzed against the same buffer until the final urea concentration was below 5 mM. The dialyzed material was centrifuged. The supernatant contained the GST-NP1 or GST-NP2 proteins and then applied to the top of a column. After the 1M NaCl elution, the column was eluted by removing calcium with EDTA (2). To search for less abundant proteins that might bind to taipoxin columns, we used a calcium-dependent method. Sequence data were searched against GenBank™ and Swiss-Prot databases.

**RESULTS**

**Chromatography on Taipoxin, rTCP49, GST-NP1, and GST-NP2 Columns Reveals a Novel Protein of 55–65 kDa**—Chromatography of solubilized brain membranes on taipoxin columns greatly enriches NP1 and TCBP49, which can be eluted by removing calcium with EDTA (2). To test for less abundant proteins that might bind to taipoxin columns, we repeated the chromatography of solubilized brain membranes on a taipoxin column in the presence of 1 mM calcium and eluted with a NaCl gradient (200 mM, 400 mM, 600 mM, 800 mM, 1 M) in 20 mM Heps, pH 7.4, 1 mM calcium chloride, 0.2% Triton X-100. After the 1 M NaCl elution, the column was eluted with 1 M NaCl containing 10 mM EDTA to disrupt interactions dependent on divalent cations. Starting membranes, column flow-through, and a similar proportion of each column fraction were run on SDS-PAGE gels. Gels were stained with Coomassie or transferred to nitrocellulose and incubated with antibodies to NP1, NP2, and TCBP49 (Fig. 1). TCBP49 elutes in the 200 and 400 mM NaCl steps, while NP1 elutes in the final 10 mM EDTA step, suggesting that TCBP49 and NP1 have different affinities for taipoxin. Calcium-dependent chromatography of recombinant TCBP49 on taipoxin columns also enriches rTCP49, indicating that TCBP49 does bind to taipoxin in the absence of NP1 (data not shown). We had previously suggested that NP2 was not enriched on taipoxin columns (6), based on the lack of immunoreactivity with an antibody to apexin (7, 8), the probable guinea pig homolog of NP2. We reinvestigated the potential enrichment of NP2 on taipoxin columns with an antibody specific to the carboxyl terminus (residues 410–430) of rat NP2/narp (9). Like NP1, NP2 is greatly enriched on taipoxin columns, is not eluted off taipoxin columns with 1 M NaCl, but is eluted with EDTA (Fig. 1). In addition to NP1 and NP2, this fraction also contains proteins that run as two diffuse bands at 55–65 kDa. Preliminary peptide analysis suggested that this was in fact a novel protein, which we have now named neuronal pentraxin receptor or NPR. In Fig. 1, these protein bands have been stained with antibody NPR1 described below. Similar chromatography on Sepharose (Fig. 1) or Tris-conjugated CH-Sepharose (2) fails to enrich NP1, NP2, NPR, or TCBP49.
To investigate potential interactions between these taipoxin-binding proteins, we performed similar chromatographies on columns of immobilized taipoxin, rTCBP49, GST-NP1, and GST-NP2. We expressed TCBP49, NP1, and NP2 in bacteria so that these proteins could be independently purified. Recombinant TCBP49 contains residues 23–318, deleting the signal peptide. We expressed NP1 and NP2 as glutathione S-transferase fusion proteins to aid in solubility. GST-NP1 and GST-NP2 proteins contain residues 19–432 of NP1 and residues 16–430 of NP2 fused at their amino termini to glutathione S-transferase. Purified rTCBP49, GST-NP1, and GST-NP2 were coupled to activated CH-Sepharose 4B according to commercial protocols. Solubilized rat brain membranes were chromatographed over these columns in the presence of 1 mM free calcium under the same conditions as the chromatography on taipoxin columns. One major protein and several minor proteins in the 10 mM EDTA eluate are prominent by Coomassie Blue staining. The major protein is NP1 based on its size and immunoreactivity with three different NP1 antibodies (Fig. 1 and data not shown). This supports a direct calcium-dependent interaction between TCBP49 and NP1 in the absence of taipoxin. Chromatography on rTCBP49 also results in substantial enrichment of NP2 and multiple NPR bands, which elute with 10 mM EDTA. While we are looking at interactions facilitated by calcium, we cannot rule out that these interactions may also be mediated by other divalent cations.

Chromatography on GST-NP1 and GST-NP2 also results in the enrichment of NP1, NP2, and NPR in the 10 mM EDTA eluate (Fig. 1). TCBP49 is only slightly enriched by chromatography on GST-NP1 and GST-NP2 columns. We expressed NPR in bacteria so that these proteins could be independently purified. NPR contains a stretch of sufficient length (22–27 amino acids) and hydrophobicity to encode a transmembrane domain. The amino terminus of the 55-kDa band was blocked. The 55- and 65-kDa bands were purified and separated and were each treated with CNBr to obtain peptide fragments. CNBr digestion of the 55 and 65 kDa bands yielded similar peptides, suggesting the two bands represented variants of the same protein, although each digest contained a prominent unique peptide (data not shown). The unique peptide of the digest of the 65-kDa band gave sequence (LAFLGAVI(C/S)ASPLAP). This sequence contains a stretch of sufficient length (22–27 amino acids) and hydrophobicity to encode a transmembrane domain. The amino terminus of the 55-kDa band was blocked. The 55- and 65-kDa bands were purified and separated and were each treated with CNBr to obtain peptide fragments. CNBr digestion of the 55 and 65 kDa bands yielded similar peptides, suggesting the two bands represented variants of the same protein, although each digest contained a prominent unique peptide (data not shown).

The longest cDNA obtained was approximately 5.5 kb in length and contained an open reading frame of about 1.5 kb (Fig. 2, GenBank™ number AF005099). As stated above, we named the protein(s) encoded by this cDNA neuronal pentraxin receptor or NPR. This cDNA does not contain an in-frame stop codon in the 147-nucleotide 5′–untranslated region. The determined amino-terminal amino acid sequence of the 65-kDa protein matches the deduced amino acid sequence of the open reading frame except that the initial amino acid of the determined sequence is a methionine and in the deduced sequence is a leucine. This suggests that the CTG at nucleotide 148 is used as an initiator codon and codes for a methionine. The CTG at nucleotide 148 is present in all clones analyzed and is present in cDNA of mouse NPR, which has 96% amino acid identity with rat NPR (data not shown). The open reading frame contains all determined amino acid sequence from both the 55- and 65-kDa proteins. Additionally, an antibody (NPR1) to the car...
Fig. 2. Nucleotide and translated amino acid sequences of message for rat NPR. The sequences are numbered to the right, with the translated amino acid sequence being shown in single-letter code below the nucleotide sequence. The amino acid sequences obtained from protein fragments of a CNBr digest are underlined. The putative transmembrane domain is indicated by the dashed underlined and is contained within determined amino acid sequence. The deduced amino acid sequence is 494 residues long (Fig. 2). Hydropathy analysis reveals a single stretch of amino acids of sufficient length and hydrophobicity to encode a transmembrane domain. This stretch of 22–27 residues starts with the third residue of the protein. The deduced amino acid sequence of NPR contains three potential N-linked glycosylation sites at residues 42, 211, and 457.

We searched SWISS-PRO and GenBank™ data bases with the deduced amino acid sequence for NPR. The sequence of NPR has significant homology to the pentraxins and to neuronal pentraxin 1 and 2 in particular (Fig. 3). NPR has 49% amino acid sequence identity with rat NP1 and 48% identity with human NP2. As with NP1 and NP2, the carboxyl-terminal half of NPR has homology (22–25% identity) with the acute phase pentraxins C-reactive protein and serum amyloid P protein (20, 21). NPR's homology to neuronal pentraxins 1 and 2 is greatest in the carboxyl-terminal half of the protein (22–25% identity) but is still significant in the amino-terminal half of the protein (~30% identity). The homology includes the putative calcium binding residues (22), the pentraxin signature sequence (HXCXSVXSW (23)), and one glycosylation site. It has been suggested that larger pentraxins bind ligands via their more conserved carboxyl-terminal halves and that their amino-terminal halves confer unique functions upon these proteins (24). If this proves to be correct, NP1, NP2, and NPR may have similarities in ligand binding and distinct functions mediated by their amino-terminal domains.

NPR Message Is Brain-specific and Localized to Similar Brain Regions as NP1 and NP2—To determine the tissue distribution of NPR message, we probed Northern blots of rat tissue poly(A)⁺ RNAs with a labeled random-primed NPR cDNA probe (Fig. 4). This NPR probe hybridizes to a single 5.5-kb mRNA. Overnight exposure of Northern blots reveals strong signal in brain and cerebellum lanes. This indicates that NPR mRNA is moderately abundant in neuronal tissues. One-week exposure of Northern blots reveals minimal or no signal in lanes of kidney, skeletal muscle, heart, and liver poly(A)⁺ RNAs, suggesting that NPR expression is highly brain specific. NPR cDNA probe also hybridizes to a 5.5-kb mRNA in nerve growth factor-induced and non-induced PC12 cells.

We investigated the cellular distribution of NPR message in the brain by in situ hybridization (Fig. 5). The highest hybridization is in the cerebellum and hippocampus. Like NP1, hybridization to NPR message is abundant in the Purkinje and granule neurons of the cerebellum and in CA3 neurons of the
It is present at moderate levels in dentate gyrus and CA1 neurons. Hybridization in the cerebral cortex is highest in layer 6 but is present diffusely in other layers. Hybridization to NPR probes is also present in the piriform cortex, taenia tecta, and cingulate cortex. There is a lack of hybridization in many midbrain regions, although at this level of detection we cannot rule out that these neurons may express low levels of NPR message. The pattern of hybridization to NPR message in the brain is similar to that of NP1 (2) and NP2 (9), although there are apparent differences in the pattern of hybridization between CA3 and CA1 regions of the hippocampus.

Hybridization to NP1 probe is highest in CA3 and very low in CA1 (2). Hybridization to NPR probe is highest in CA3 but is still substantial in CA1. Reports of the hybridization of NP2 probe seem to suggest similar amounts in CA3 and CA1 (9).

**Fig. 3.** Alignment of the amino acid sequence of NPR with NP1 and NP2. The sequences are numbered on the right. Identical residues are boxed. Putative calcium binding residues are shaded. The pentraxin signature sequence is marked with an overlying bar. RNPR, rat neuronal pentraxin receptor; RNPI, rat neuronal pentraxin 1 (2); RNPII, human neuronal pentraxin 2 (6).

**Fig. 4.** RNA blot analysis of message for NPR in rat tissues. Poly(A)⁺ enriched RNA (5 µg) from rat brain, cerebellum, kidney, skeletal muscle, heart, liver, and testis, as well as the rat neurosecretory cell line PC12 (uninduced and nucleic acid growth factor-induced) were electrophoresed, blotted, and probed with a ³²P-labeled fragment of NPR cDNA (nucleotides 910–1241). The top/left panel is a 1-day autoradiographic exposure, the top/middle panel is 7 days, the two top/right panels are 13 days (uninduced) and 7 days (induced), respectively. The bottom panels show hybridization of the same Northern to a cDNA probe to glyceraldehyde phosphate dehydrogenase (GAPDH).

**Fig. 5.** Distribution of NPR message in brain sections revealed by in situ hybridization. Representative 7-µm sections of rat brain were hybridized to ³²P-UTP labeled NPR-riboprobes as outlined under “Experimental Procedures.” Hybridization (detection of silver grains in the emulsion) is shown for a dark field image. NPR message is in the cerebellum (A), hippocampus (B), cerebral cortex (C), cingulate cortex (D), and taenia tecta (E). In the cerebellum hybridization is seen in Purkinje and granule neurons. In the hippocampus there is strong hybridization in CA3, moderate in the CA1, and low in the dentate gyrus (DG). In the cortex, hybridization is strongest in layer 6 of the neocortex and layer 2 of the olfactory cortex. Nuclei staining is not shown.

**Fig. 6.** Deglycosylation of NPR by endoglycosidase F/glycopeptidase F. Endoglycosidase F/glycopeptidase F treatment reduces the apparent molecular weight of all NPR immunoreactive proteins and only a small proportion is reduced to the size of recombinant NPR (residues 30–494). Aliquots of a column fraction containing NPR were incubated overnight at 37 °C in the absence (NPR o/n) or presence (NPR/endo F) of a mixture of endoglycosidase F/glycopeptidase F. Native NPR or recombinant NPR was used as controls. Samples were run on SDS-PAGE gels, transferred to nitrocellulose, and blotted with either NPR1 (recognizes carboxyl terminus of NPR) or NPR2 (recognizes post-transmembrane region of NPR) antibodies.
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sisting of the entire NPR sequence after the apparent transmembrane domain (residues 30–494) and included this protein on the same gels. Proteins were transferred to nitrocellulose and stained with NPR antibodies (Fig. 6). Blots were incubated with an antibody NPR1 that recognizes the carboxyl terminus (residues 472–494 of NPR) or an antibody NPR2 that recognizes residues immediately after the transmembrane sequence (residues 31–57 of NPR). Additionally, the peptide sequence recognized by NPR2 contains one of the three putative N-linked glycosylation sites of the protein (residue 42). 55- and 65-kDa NPR bands are recognized by both antibodies. However, while NPR1 appears to recognize 55- and 65-kDa bands equally well, NPR2 recognizes the 55-kDa protein well, but only very poorly recognizes the 65-kDa protein. This may suggest that the 65-kDa protein is glycosylated at residue 42, while the 55-kDa protein is not. Both antibodies strongly recognizes the 55-kDa protein, suggesting that this apparently smaller protein contains the full deduced amino acid sequence of NPR carboxy-terminal to the proposed transmembrane domain. On incubation with endoglycosidase F, both bands shift to lower molecular weights, suggesting that NPR does contain N-linked glycosylation and consequently that all forms proceed through the secretory pathway. However, NPR immunoreactivity remains diffuse and appears to contain several distinct bands ranging from approximately 50 to 60 kDa in apparent molecular weight.

The multiple forms of NPR observed both before and after endoglycosidase F treatment do not appear to represent separate genes or alternative splicing, as we have identified only one type of cDNA and see only one message in Northern analysis. Additionally, the two major forms observed before endoglycosidase F treatment yield similar proteolytic products and apparently all forms are recognized by both carboxy-terminal (NPR1) and post-transmembrane domain (NPR2) antibodies. As discussed, multiple forms of NPR are likely to be due in part to differential glycosylation; however, they may also represent proteolytic cleavage of the transmembrane domain or use of alternative initiation sites. In other proteins initiating with CTG, it has been found that this initiator codon is actually used as an alternative to a downstream ATG initiation site (25). Although no ATG codons are present in the 5'-untranslated region of the NPR cDNA, a downstream ATG at nucleotide 181 (methionine 12) could function as an initiation codon. NPR, if initiated at methionine 12, would have a hydrophobic domain of 13–19 residues that is bounded only carboxy-terminally with charged residues. At present, additional experiments are necessary to determine if variability in the molecular weight of NPR is due to use of alternative initiation sites, proteolytic cleavage of the putative amino-terminal transmembrane domain, or other post-translational modifications.

DISCUSSION

We have previously identified two major taipoxin-binding proteins, NP1 and TCBP49 (2, 3), and suggested that they mediate the uptake and activation of taipoxin, a presynaptic-acting neurotoxin that blocks synaptic vesicle recycling (1, 2). We have shown that the addition of NP1 to glial cultures renders them susceptible to taipoxin toxicity (2), supporting a direct interaction between taipoxin and NP1 and a role for NP1 in the uptake of taipoxin. Additionally, the enrichment of rTCBP49 on taipoxin columns suggests that TCBP49 also interacts directly with taipoxin (data not shown). We now show that these two taipoxin-binding proteins are capable of binding to each other in the absence of taipoxin. Based on the homology of NP1 to acute phase pentraxins proposed to be involved in the uptake of pathogens and cellular debris, we have hypothesized that these proteins are components of a novel neuronal uptake pathway responsible for the clearance of synaptic debris and the uptake of taipoxin. Here we identify additional proteins that are likely constituents of this pathway. These include the previously characterized pentraxin, NP2, and a novel putative integral membrane pentraxin that we have named NPR.

Chromatography of solubilized brain membranes on columns of immobilized taipoxin greatly enriches NP1, TCBP49, and 2–3 minor proteins of apparent molecular masses between 50 and 65 kDa. One of these minor proteins is neuronal pentraxin 2 or NP2 (6). NP2 was identified from brain cDNA libraries by low stringency screening with NP1 probes and has also been identified as a guinea pig sperm acrosomal protein, apexin/p50 (7, 8), and as a neural activity regulated pentraxin, narp (9). With specific antiserum to rat NP2, we show that NP2 is also enriched on taipoxin columns. An additional minor protein enriched on taipoxin columns runs at both 55 and 65 kDa, and we now characterize it as NPR.

Northern analysis and in situ hybridization show that NPR is expressed in neurons. NPR is expressed in the same brain regions that express NP1 and NP2. All show the highest message expression in cerebellum and hippocampus and lower expression in the neocortex and piriform cortex. The overlap of expression of message for NP1, NP2, and NPR in brain may reflect a functional interaction between these proteins. Message for the apparent rat homolog of NP2, narp, has been shown to be greatly up-regulated with high frequency stimulation of the hippocampus (9). Potential activity-dependent regulation of NPR or NP1 has not been investigated; however, NPR and NP1 normally have moderately abundant messages of 5–6 kb, while NP2 has a low abundance message of 2.5 kb.

NPR protein, like NP1 and NP2, is rare. Two different NPR antibodies fail to detect NPR in cortical and cerebellar homogenates or in brain membranes. The rarity of NPR protein contrasts with the abundance of NPR message. This discrepancy can be explained if NPR has a rapid turnover. Antibodies specific to the carboxyl terminus and post-transmembrane portion of NPR recognize both the 55- and 65-kDa NPR proteins. This demonstrates that all NPR proteins contain residues 30–494. The inability of antibody NPR2 (raised to a peptide containing a glycosylation site) to recognize the multiple forms of NPR equally well suggests that some of the NPR diversity is due to differential glycosylation. However, deglycosylation of NPR with endoglycosidase F yields multiple proteins of 50–60 kDa, suggesting that multiple NPR forms are not generated solely by differential glycosylation. Edman degradation of the 65-kDa NPR yielded clear sequence whereas Edman degradation of the 55-kDa NPR was blocked, suggesting that the 55- and 65-kDa NPR proteins have different amino termini. If NPR proteins differ in the first 30 residues, such differences are likely due to differential use of initiator codons or proteolytic cleavage. If initiated at methionine 12, NPR would have an amino-terminal hydrophobic domain of 13–19 residues. We currently cannot determine if the 55-kDa form of NPR contains residues 1–30, but endoglycosidase F cleavage produces only a small proportion of protein that runs at a similar molecular weight as recombinant NPR engineered to lack residues 1–30, its transmembrane domain. Expression of NPR in transfected CHO cells leads to cell surface exposure but not secretion.2 suggesting that transfected NPR contains a transmembrane domain.

NPR exhibits several properties consistent with a role in uptake of NP1 and NP2. First, NPR, in at least the 65-kDa form, has an uncleaved amino-terminal sequence of sufficient length and hydrophobicity to be a transmembrane domain. This putative transmembrane domain and the presence of N-

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linked glycosylation are consistent with NPR being a cell surface integral membrane protein. Second, NPR can interact with NP1 and NP2, as NPR is greatly enriched on columns of recombinant NP1 and NP2. This interaction is direct as NPR, NP1, and NP2 are the major proteins that remain bound to GST-NP1 and GST-NP2 columns in high salt concentrations. The interactions of NPR, NP1, and NP2 are likely to occur through their calcium-dependent ligand binding domains, as these proteins are specifically eluted with EDTA. Third, the pattern of expression of NPR message in the brain overlaps that of NP1 and NP2. All are expressed most highly in the cerebellum and hippocampus with lower amounts in the neocortex. Key to the hypothesis that NPR is a receptor for secreted NP1 and NP2 will be the demonstration that transfection of NPR into cell lines allows cells to take up NP1, NP2, and taipoxin-NP1 complexes. Transfection of NPR will also allow investigation of functions of NPR that are independent of NP1 and NP2 and whether some forms of NPR are secreted.

The interaction of TCBP49 with these neuronal pentraxins in the absence of taipoxin suggests that TCBP49, NP1 NP2 and NPR are components of a common pathway. Their co-enrichment on taipoxin columns suggests that this pathway is responsible for taipoxin toxicity and, as we have discussed, may provide a mechanism for the uptake of taipoxin into synapses. The pentraxins seem well suited to mediate internalization, and we have proposed that the interaction between TCBP49 and taipoxin may mediate toxicity, possibly by activating taipoxin after it has been taken up into a luminal TCBP49-containing compartment. TCBP49's six EF-hands and high affinity calcium binding suggest that TCBP49 mediates calcium regulation of luminal, reticular processes. Such processes could include protein folding, sorting, and membrane trafficking and may have direct implications for the action of taipoxin in blocking synaptic vesicle recycling. It is also interesting to note that the human homolog of TCBP49, ERC55 (12), has been identified by a two-hybrid screen as a ligand for the human papillomavirus E6 oncoprotein (26), raising a possibility that TCBP49 is involved in regulating membrane traffic necessary for growth control.

The identification of NP1, NP2, and NPR has defined a specific family of pentraxins. All three have approximately 50% identity to each other, and each has 20–25% identity to the smaller acute phase pentraxins, C-reactive protein, and serum amyloid P protein. Additionally, experiments have suggested that NP1, NP2, and NPR can exist as high molecular weight complexes, indicating that they may form pentamers or decamers like C-reactive protein and serum amyloid P protein (data not shown). C-reactive protein and serum amyloid P protein bind a wide variety of materials, including phosphorylcholine, phosphorylethanolamine, particular bacterial polysaccharides, terminal galactose residues, 3-sulfated sugars, chromatin, histones, DNA, and components of the complement pathway (4, 5). It has been proposed that the diverse binding abilities of these proteins and their elevation during acute phase allow the clearance of extracellular material during immune response (4, 5). We suggest that NP1, NP2, and NPR mediate a similar function for neurons. Such a function would be ideally suited for removal of degraded synaptic material generated as synapses are formed or remodeled. Further studies will be required to test whether these neuronal pentraxins bind to degraded neuronal or synaptic material and whether loss of expression of these neuronal pentraxins affects synaptogenesis or plasticity.

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