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A 38,000-dalton membrane protein (p38) present in synaptic vesicles

(synapsin I/subcellular fractionation/integral membrane protein)

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ABSTRACT **A protein with an apparent molecular mass of 38,000 daltons designated p38 was found in synaptic vesicles from rat brain. The subcellular distribution of p38 and some of its properties were determined with the aid of polyclonal and monoclonal antibodies. The subcellular distribution of p38 was similar to that of synapsin I, a synaptic-vesicle specific phosphoprotein. p38 in the synaptic vesicle fraction purified by controlled-pore glass bead chromatography showed an enrichment of more than 20-fold over the crude homogenate. Immunostaining of sections through various brain regions revealed an intense labeling of most, and possibly all, nerve terminals. Only faint reaction in the region of the Golgi apparatus and no detectable labeling of axons and dendrites was observed. Two-dimensional electrophoresis revealed that p38 has an acidic pI. Solubilization experiments, as well as phase separation experiments using Triton X-114, indicated that p38 is an integral membrane protein. Binding of antibodies to intact synaptic vesicles, as well as controlled proteolytic digestion of intact and detergent-treated vesicles, revealed that p38 has a domain exposed on the cytoplasmic surface.**

Mammalian brain synaptic vesicles have some unique functions in common, despite their heterogeneity with respect to neurotransmitter content and morphological appearance. They store neurotransmitters in the nerve terminal, move to the presynaptic membrane, and release neurotransmitters by exocytosis upon electrical stimulation (for review, see ref. 1). These functions most likely require a specific set of proteins. However, the molecular components involved in exocytosis have not yet been elucidated. Acetylcholine-containing vesicles, which can be obtained as a homogeneous population in large yields and high purity from the electric ray (*Torpedo* and *Narcine*), have been studied in great detail (for reviews, see refs. 2–4). They contain 6–8 major protein bands (5–7) and a proteoglycan-like component (8, 9), most of which are apparently specific for these vesicles. The protein composition of mammalian brain synaptic vesicles is less well known. Two proteins specific for synaptic vesicles have been characterized so far: synapsin I, a major phosphoprotein doublet of 80–86 kDa (10–13) and a protein of 65 kDa (14). Synapsin I is a neuron-specific protein found in all nerve terminals studied (10, 11). Synapsin I is bound to the cytoplasmic surface of small synaptic vesicles (11), from which it can easily be extracted by high salt concentrations (12). It is apparently absent from large dense-core (probably peptidergic) vesicles (15). The 65-kDa protein was detected by examining monoclonal antibodies prepared against synaptic junctional complexes (14). Two of those antibodies recognized this vesicle-specific protein in mammalian brain (14). In contrast to synapsin I, the 65-kDa protein is apparently an intrinsic membrane protein.

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We now report a new vesicle-specific protein of 38-kDa which is apparently a major component of all brain synaptic vesicles.

MATERIALS AND METHODS

Materials. Nitrocellulose membrane filters (pore size, 0.20 and 0.10 μm) were obtained from Schleicher & Schuell. ^{125}I-labeled protein A (70–100 mCi/mg; 1 Ci = 37 GBq) was from New England Nuclear. Affinity-purified goat anti-rabbit IgG and goat anti-mouse IgG, coupled to horseradish peroxidase, were from Bio-Rad. Ampholines (pH 3.5–10 and pH 5–7) were from LKB. Trypsin (bovine pancreas, type III) was from Sigma, and pronase (B grade) was from Calbiochem. All other reagents were of analytical grade and from commercial sources. Synapsin I (protein I), prepared from bovine brain by a modification of the procedure of Ueda and Greengard (13), and rabbit antiserum raised against purified synapsin I were gifts of Jesse Chan and Wilson Wu.

Isolation of Synaptic Vesicles. Isolation of synaptic vesicles was performed by the procedure of Nagy *et al.* (16) as adapted by Huttner *et al.* (12) with minor modifications. All procedures were carried out at 4°C. Briefly, cerebral cortices of 14 rats were homogenized in 150 ml of 0.32 M sucrose containing 0.1 mM phenylmethylsulfonyl fluoride by 10 strokes at 900 rpm in a glass/teflon homogenizer. The homogenate was spun for 10 min at 800 × *g* to yield a pellet (designated P_1) and a supernatant (designated S_1). S_1 was then centrifuged for 15 min at 9,200 × *g*. The resulting pellet (P_2') was washed once in 120 ml of 0.32 M sucrose, yielding the crude synaptosomal pellet P_2. The combined supernatants (referred to as S_2) were centrifuged at 120,000 × *g* for 1 hr, yielding pellet P_3 and supernatant S_3.

The crude synaptosomal pellet P_2 was resuspended in 12 ml of 0.32 M sucrose and lysed by rapid addition of 9 vol of distilled water followed by homogenization in a glass/teflon homogenizer (three strokes at 2,000 rpm). Hepes/NaOH buffer (pH 7.4) was then added to a final concentration of 10 mM. After incubation on ice for 30 min, the lysed synaptosomes were spun at 25,000 × *g* to yield a lysate pellet (LP_1) and a lysate supernatant (LS_1). LS_1 was then centrifuged for 2 hr at 165,000 × *g* to yield a pellet (LP_2) and a supernatant (LS_2). LP_2 was resuspended in 4 ml of 40 mM sucrose and layered on top of 55 ml of a continuous sucrose gradient (50–800 mM sucrose). After centrifugation for 5 hr in a Beckman SW 25.2 rotor at 65,000 × g_{avg}, the gradient was collected in four fractions: the vesicle-containing band between 200 and 400 mM sucrose (SG 2), the fractions on top (SG 1) and underneath (SG 3), and the pellet fraction (SG 4). SG 2 was then loaded onto a column filled with 400 ml of glyceryl-coated controlled-pore glass beads (CPG) with a mean pore diameter of 3000 Å (120/200 mesh) and was eluted with 0.3 M glycine buffered with 10 mM Hepes/NaOH at pH 7.4. If not indicated otherwise, the fractions corresponding to the second peak were pooled, centrifuged for 2 hr at 165,000

Abbreviation: CPG, controlled-pore glass bead(s).

× *g* and resuspended in ≈1 ml of glycine buffer, this fraction being referred to as "purified synaptic vesicles." (For fractionation scheme, see ref. 12.) The volumes of all fractions were determined (the pellets after resuspension in 0.3 M sucrose), and aliquots were frozen in liquid nitrogen for the estimation of protein, p38, and synapsin I.

Generation of Serum Antibodies. Monospecific antisera against p38 were prepared as follows. Purified synaptic vesicle protein (3 mg) was separated by $NaDodSO_4$/polyacrylamide gel electrophoresis (150 μg per lane, see below). The bands were visualized with sodium acetate on the unfixed gel (17). Since p38 is a major band (see Fig. 1), it could be located readily. In one procedure, the p38 band was cut out and used directly for immunization after homogenization of the gel piece. In a second procedure, the protein was eluted from the gel and precipitated as described (18), and the pellet was resuspended in physiological saline. The samples were emulsified in complete Freund's adjuvant and injected intradermally at multiple sites into New Zealand female rabbits. Booster injections containing one-third of the original amount were given at weeks 1, 2, and 3 after the initial injection. The animals were bled 1 week after administration of the final booster. The two procedures yielded antisera of approximately the same specificity and titer.

Generation of Monoclonal Antibodies. NS 1 myeloma cells were obtained as a gift from C. Barnstable (The Rockefeller Univ.) and later grown from our own frozen stocks. BALB/c mice (4–6 weeks old) were injected intraperitoneally with purified synaptic vesicles (50 μg of protein) mixed with complete Freund's adjuvant (final vol. 0.5 ml), and boosters containing 30 μg of protein mixed with incomplete Freund's adjuvant were injected intraperitoneally 3 weeks later. Four weeks later, 15 μg of vesicle protein, resuspended in 150 mM NaCl, was injected intravenously. Three days later, the fusion was performed according to standard procedures (19, 20). After the fusion, the cells were plated on microtiter plates at 2×10^5 cells per well, with peritoneal macrophages (10^4 cells per well) as the feeder layer (20). After hybrid cells covered one-fourth to one-third of the well, 150 μl each of the supernatants were withdrawn, diluted with 100 μl of 150 mM NaCl, and used for screening in an immunoblot assay as described below. Positive colonies were expanded to 1-ml cultures, cloned, rescreened, and recloned by established procedures (20, 21). Ascites tumors were generated in pristane-primed BALB/c mice (22). For the experiments described in this paper, the ascites fluid was freed from its cellular components by centrifugation and used without further purification.

Proteolytic Digestion of Vesicles. The incubation mixture contained 400 μg of purified synaptic vesicles per ml, 250 mM glycine, 8 mM Hepes/NaOH (pH 7.2), 30 mM NaCl, 1% (vol/vol; only if indicated) Triton X-100, and 50 μg of trypsin or 10 μg of Pronase per ml in a final volume of 0.3 ml. The incubation, started by the addition of the protease, was carried out at 22°C. At the times indicated, aliquots of 30 μl (60 μl for gels to be silver-stained) were removed, added to 30 μl of 4% (wt/vol) $NaDodSO_4$, boiled immediately for 2 min, and then stored in liquid nitrogen until used for electrophoresis and immunoblotting.

Electrophoresis and Immunoblotting. Nonequilibrium pH gradient electrophoresis was performed as described by O'Farrell *et al.* (23) with gels of 2.5-mm diameter. $NaDodSO_4$/polyacrylamide gel electrophoresis was carried out (24) with gels of 1.5-mm thickness and 12-cm length. Silver staining (25) and transfer to nitrocellulose (26) were carried out as described. Immunolabeling was performed in one procedure by using ^{125}I-labeled protein A (27), and visualization was by autoradiography. Immunolabeling was performed in a second procedure by using a commercially available horseradish peroxidase-coupled secondary antibody dissolved in a buffer containing Tween 20 (28); in this case, the bands were visualized with 4-chloro-1-naphthol (29). For screening of hybridoma supernatants, small gels (6 × 8 cm, 0.75 mm thick) were run by using the Bio-Rad mini-vertical-slab cell (model 360). Vesicle proteins (2.5 μg per lane) were separated by $NaDodSO_4$/polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The membranes were cut into strips, and each strip was fixed with 10% (vol/vol) acetic acid/25% (vol/vol) isopropanol, washed with 150 mM NaCl/10 mM potassium phosphate buffer, pH 7.2, incubated for 1 hr with hybridoma supernatant, and visualized with horseradish peroxidase-conjugated goat anti-mouse IgG.

Other Analytical Procedures. Quantitation of p38 and synapsin I was performed as described (27). p38 was measured by using purified synaptic vesicles as the protein standard; 1 unit of p38 was defined as the amount found in 1 μg of standard protein. The relative enrichments of p38 and synapsin I were obtained by determining the ratio of the amount per mg of protein found in the subfraction to the amount per mg of protein found in the homogenate. Protein was determined by the method of Bradford (30) and by a modification (31) of the method of Lowry *et al.* (32). The two methods gave similar results. Partition of vesicle proteins into detergent-binding and nonbinding proteins was performed with Triton X-114 as described (33).

Immunocytochemistry. Rats were perfused with 500 ml of the zinc/formaldehyde fixative of Mugnaini and Dahl (34). Brains were removed 1 hr after fixation *in situ* and cut into 25- to 50-μm slices with a Vibratome. The slices were washed four times (15 min each) in 0.5 M Tris chloride (pH 7.6) and were incubated in ascites containing monoclonal antibody C 7.1 (dilution, 1:2000) overnight, in rabbit anti-mouse IgG (dilution, 1:40) for 1 hr, and mouse peroxidase-anti-peroxidase (dilution, 1:100) for 1 hr. Each antibody incubation was followed by three 10-min washes in 0.5 M Tris chloride. The antibodies were diluted in 0.5 M Tris chloride with 0.25% (wt/vol) carrageenan added to reduce background as described by Renfroe *et al.* (35). The sections were incubated for 5 min in a solution containing 0.05% diaminobenzidine, 0.01% H_2O_2, and 0.02% $CoCl_2$ as described by Rye *et al.* (36). Controls included the omission of each of the immunoreagents and the substitution of normal mouse serum and of monoclonal antibodies against other proteins of known distribution. The controls indicated that the staining for p38 was specific.

RESULTS

Subcellular Localization of p38. The amounts of p38 and synapsin I present in various subcellular fractions are shown in Table 1. The two proteins were distributed in a parallel manner among almost all of the subfractions, with 23-fold (p38) and 18-fold (synapsin I) enrichments over the crude homogenate, in the peak fractions eluted from the CPG column. Fig. 1 shows the pattern of selected subfractions analyzed by electrophoresis and immunoblotting. A single band was recognized by the antiserum in all subfractions.

In order to study the relationship between the localization of p38 and synapsin I further, the amounts of the two proteins were determined in fractions obtained after CPG-chromatography. Both proteins comigrated only with the second (synaptic vesicle) peak (Fig. 2). The first peak [consisting mainly of membrane sheets of equal density (see ref. 12)], was virtually free of p38 and synapsin I.

In addition to the antisera described, four monoclonal antibodies were obtained (C 7.1, C 7.2, C 7.3, and C 7.4) that reacted with p38. Preliminary experiments with these antibodies yielded results similar to those obtained with the antiserum (not shown).

Table 1. Total recoveries and relative enrichments of p38 and synapsin I in subcellular fractions

	Total recovery, %		Relative enrichment	
	p38	Synapsin I	p38	Synapsin I
Homogenate	100	100	1.00	1.00
P_1	17.9	16.8	0.69	0.65
S_1	68.0	66.5	1.00	0.97
P_2	22.2	22.7	0.95	0.97
S_2	36.3	34.3	0.72	0.67
P_3	35.1	40.2	1.28	1.44
S_3	1.1	1.4	0.06	0.08
LP_1	14.8	15.4	1.07	1.27
LS_1	7.4	7.0	0.62	0.57
LP_2	7.5	5.2	5.68	3.64
LS_2	0.1	0.1	0.02	0.02
SG 1	0.07	0.15	0.20	0.44
SG 2	2.58	2.53	8.08	7.92
SG 3	0.47	0.76	3.47	5.69
SG 4	1.05	1.32	4.68	5.69
CPG-column peak fractions	—	—	23.4	18.0

Quantitation of p38 and synapsin I was performed as described (27). The values are the means of results obtained in two fractionation experiments.

Fig. 3 shows a coronal section through the midbrain, immunostained with monoclonal antibody C 7.1. Immunoreactive puncta, shown by electron microscopy to be nerve terminals (data not shown), surround unstained somata and dendrites. Axons and glial cells also were unstained. These results correspond well with the known distribution of synapsin I as described earlier (10). In addition, faint perinuclear staining (probably of Golgi apparatus) was observed in the perinuclear cytoplasm of some neurons, which was not seen with immunostaining for synapsin I.

Two-Dimensional Electrophoresis and Extraction Properties of p38. p38 was further characterized by two-dimensional electrophoresis followed by protein staining (Fig. 4*A*) or immunoblotting (Fig. 4*B*). A single band at the acidic end of the gel, corresponding to a major protein band on the silver-stained gel, was immunoreactive.

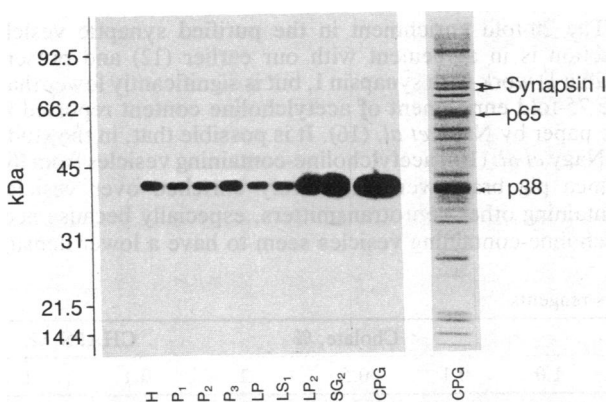

FIG. 1. (*Left*) Immunoblots of various subcellular fractions using a rabbit antiserum against p38. Lanes: H, homogenate; CPG, peak fraction of the CPG-column eluate; others, fractions (pellets and supernatant designations) as described in *Materials and Methods*. Each subfraction (5 μg) was separated on 7.5–15% $NaDodSO_4$/polyacrylamide gradient gels and transferred to nitrocellulose membranes (0.2-μm pore size). The nitrocellulose membranes were incubated with antiserum (dilution, 1:300) and visualized by the protein A procedure. Right: Protein staining pattern (Coomassie blue) of the CPG-column.

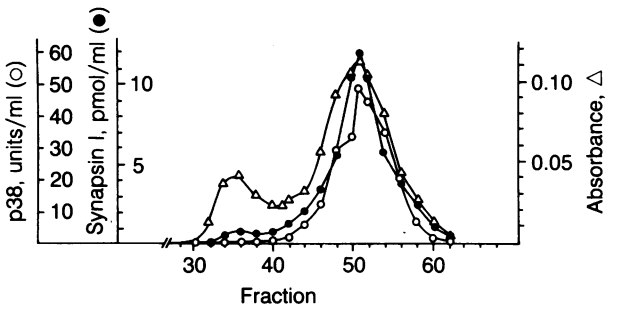

FIG. 2. Elution profile for p38, synapsin I, and total protein absorbance from the CPG-column (5.5 ml per fraction). The absorbance was corrected for light scattering by subtracting the readings obtained at 310 nm from the readings obtained at 280 nm.

To determine the nature of the binding of p38 to the vesicle membrane, a series of extraction experiments was carried out (Table 2). High salt concentrations, basic pH, chaotropic agents (NaBr, NaSCN) and weak, nondisruptive detergents (Tween 20, Tween 80) were unable to dissociate p38 from the vesicle pellet. However, stronger detergents of the nonionic type (Triton X-100) or ionic type (desoxycholate, Zwittergent, and to a lesser degree, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate and cholate) solubilized p38. These extraction properties contrast with the extraction properties of synapsin I, which dissociates at moderate salt concentrations. These data indicate that p38 is an integral membrane protein. In addition, the partition of p38 and synapsin I in the two-phase system of Triton X-114 and water

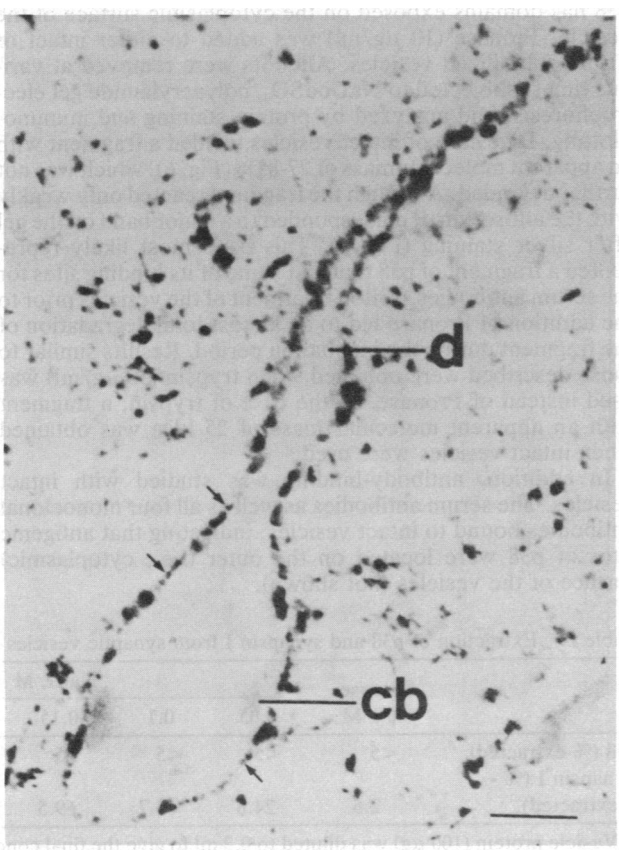

FIG. 3. A photomicrograph of immunostained nerve terminals (see, for example, small arrows) surrounding a cell body (cb) and its dendrite (d) in the rat deep mesencephalic nucleus. In addition, many strongly immunostained nerve terminals are present throughout the neuropil. Very light immunostaining is seen in the perinuclear cytoplasm. (Bar = 1 μm.)

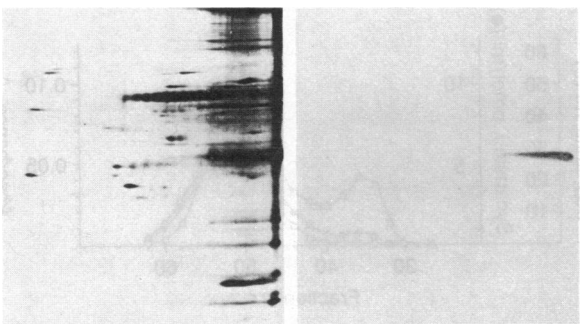

FIG. 4. Two-dimensional separation of proteins present in purified synaptic vesicles. Two identical samples (30 μg of protein each) were separated by nonequilibrium pH gradient electrophoresis in the first dimension and $NaDodSO_4$/polyacrylamide gel electrophoresis (linear gradient from 7.5% to 15%) in the second dimension. One gel was silver-stained, and the other was transferred to nitrocellulose membrane filters (0.2-μm pore size). (*A*) Silver stain of the proteins. (*B*) Immunoblot incubated with monoclonal antibody C 7.1 and visualized by the horseradish peroxidase procedure.

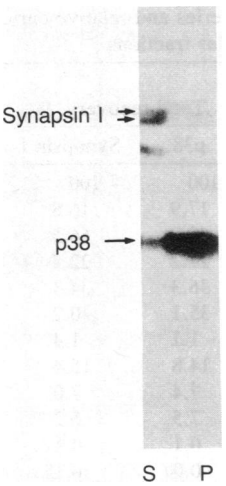

FIG. 5. Phase distribution of p38 and synapsin I in Triton X-114. A solution of 50 μg of synaptic vesicle protein was adjusted to 150 mM in NaCl and 1% (vol/vol) in Triton X-114 (final vol, 0.5 ml). After phase separation by elevating the temperature to 30°C and washing of the supernatant (33), the two phases were adjusted to equal volumes. Equal aliquots of the detergent-rich pellet and hydrophilic supernatant were run on a $NaDodSO_4$/7.5–15% polyacrylamide gradient gel, and the separated proteins were transferred to nitrocellulose membranes (pore size, 0.2 μm). The membranes were incubated with antisera against both p38 and synapsin I and subsequently were visualized by the ^{125}I-labeled protein A procedure. S, supernatant (aqueous phase); P, pellet (detergent phase). The band below the synapsin I doublet is a breakdown product of synapsin I.

was studied. This method allows the separation of proteins that bind detergents at their hydrophobic regions from proteins without hydrophobic domains (33). p38 was enriched in the detergent-rich pellet, whereas synapsin I was found almost exclusively in the hydrophilic, detergent-poor upper phase (Fig. 5).

Orientation of p38 in the Vesicle Membrane. A series of proteolytic digestions was carried out to determine whether p38 has domains exposed on the cytoplasmic surface of the vesicle. Pronase (10 μg/ml) was added to either intact or Triton-solubilized vesicles. Aliquots were removed at various times, subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis, and analyzed by protein staining and immunoblotting. Digestion of intact vesicles yielded a fragment with an apparent molecular mass of 27-kDa (Fig. 6), which was not further degraded. Although the fragment reacted only weakly with the antiserum, it corresponded to a major band on the gel after silver staining (Fig. 6). This band most likely represented a fragment of p38 that lost some of its binding sites for the serum antibodies. Triton treatment of the vesicles prior to the addition of Pronase led to an almost total degradation of the fragment during the incubation period. Results similar to those described were obtained when trypsin (50 μg/ml) was used instead of Pronase. In the case of trypsin, a fragment with an apparent molecular mass of 25 kDa was obtained when intact vesicles were used.

In addition, antibody-binding was studied with intact vesicles. The serum antibodies as well as all four monoclonal antibodies bound to intact vesicles, indicating that antigenic sites of p38 were located on the outer (i.e., cytoplasmic) surface of the vesicles (not shown).

DISCUSSION

The subcellular fractionation data presented here indicate that p38 is located specifically on synaptic vesicles. Our immunocytochemical data (Fig. 3; F. Navone and P. DeCamilli, personal communication) present clear evidence for an almost exclusive decoration of nerve terminals. Like synapsin I, p38 is exposed on the cytoplasmic side of the vesicles. In contrast to synapsin I, p38 is an integral membrane protein with an acidic isoelectric point. Based on molecular mass standards, the apparent molecular mass of p38 [previously referred to as p36 (12, 27)] was redetermined by $NaDodSO_4$/polyacrylamide gel electrophoresis to be 38 kDa.

The 20-fold enrichment in the purified synaptic vesicle fraction is in agreement with our earlier (12) and present (Table 1) work with synapsin I, but is significantly lower than the 75-fold enrichment of acetylcholine content reported in the paper by Nagy *et al.* (16). It is possible that, in the study of Nagy *et al.* (16), acetylcholine-containing vesicles from the guinea pig brain were selectively enriched over vesicles containing other neurotransmitters, especially because acetylcholine-containing vesicles seem to have a lower density

Table 2. Extraction of p38 and synapsin I from synaptic vesicles by various reagents

	Sucrose, 40 mM	NaCl, M					Cholate, %			CHAPS, %	
		0.05	0.1	0.15	0.5	1.0	0.1	0.5	2	0.1	1
p38 (% extracted)	<5	<5	<5	<5	<5	<5	<5	16.2	48.9	6.3	50.4
Synapsin I (% extracted)	2.5	24.8	65.7	69.5	81.9	93.0	>80	>80	>80	>80	>80

Vesicle protein (100 μg) was diluted to 0.2 ml to give the final concentrations indicated. Detergents, expressed as % (wt/vol), were dissolved in 150 mM NaCl. After incubation on ice for 2 hr, the samples were centrifuged for 30 min at 200,000 × *g* with a Beckman Ti 42.2 rotor. The supernatant was withdrawn, and the pellet was resuspended in 0.1% (wt/vol) $NaDodSO_4$, and p38 and synapsin I were determined in the pellet and the supernatant by the dot-immunobinding assay (27). The amount extracted was calculated on the basis of the recovered total amount. In addition to the extraction conditions shown, p38 and synapsin I were both extracted by >80% with 0.1% Triton X-100, 0.1% deoxycholate, and 0.1% Zwittergent. p38 was extracted by <5% and synapsin I by >80% with 0.3 M and 1.0 M NaBr, 0.3 M and 1.0 M NaSCN, 0.1 M Na_2CO_3, 0.2% Tween 20, and 0.2% Tween 80.

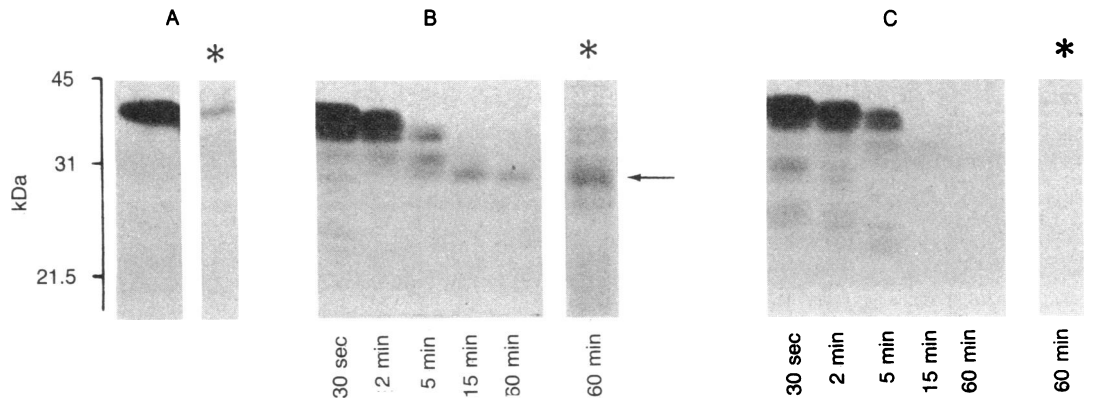

FIG. 6. Pronase digestion of intact and Triton X-100-treated synaptic vesicles. At the indicated incubation times, the reaction was stopped, and the proteins were subjected to separation on $NaDodSO_4$/14% polyacrylamide gels and transferred to nitrocellulose membranes (pore size, 0.1 μm). The bands were incubated with antiserum against p38 and visualized by the ^{125}I-labeled protein A procedure. The figure shows immunoblots and corresponding silver-stained lanes (*). (*A*) Control (no protease). (*B*) Intact vesicles digested with Pronase (10 μg/ml). (*C*) Vesicles dissolved in Triton X-100 and digested with Pronase (10 μg/ml). The arrow points to the position of a major band that appeared and remained stable only in the absence of detergent.

than vesicles containing noradrenaline or peptides (37). It cannot be ruled out, but seems highly unlikely (12), that our preparation of synaptic vesicles was contaminated significantly by other membrane vesicles.

In contrast to synapsin I, which is neuron-specific (10), preliminary experiments have shown that antisera against p38 cross-react with identical or very similar proteins in endocrine cells (adrenal medulla, anterior pituitary, endocrine pancreas, and PC 12 cells) but not with proteins in exocrine glands or any other nonneuronal tissue studied so far (F. Navone and P. de Camilli, personal communication; unpublished observations). In this regard, the distribution of p38 in extraneuronal tissue resembles that of a 65-kDa integral membrane protein (14) and possibly that of a transmembrane glycoprotein of about 100 kDa (38). These findings suggest that synaptic vesicles and endocrine secretory granules have a set of membrane proteins in common that are not shared with exocrine secretory granules. A number of other studies have dealt with the protein composition of mammalian brain synaptic vesicles (39–44). However, due to differences in methods and in purity of the vesicle fractions obtained, it is difficult to evaluate whether a protein band or protein fraction described in any of these earlier studies corresponds to p38. A more detailed study of the tissue and species distribution of p38 is under way.

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1. Reichardt, L. F. & Kelly, R. B. (1983) *Annu. Rev. Biochem.* **52,** 871–926.
2. Whittaker, V. P. (1984) *Biochem. Soc. Trans.* **12,** 561–576.
3. Kelly, R. B. & Hooper, J. E. (1982) in *The Secretory Granule*, eds. Posner, A. M. & Trifaro, J. M. (Elsevier, New York), pp. 81–118.
4. Zimmermann, H. (1982) in *Neurotransmitter Vesicles*, eds. Klein, R. L., Lagercrantz, H. & Zimmermann, H. (Academic, New York), pp. 241–374.
5. Tashiro, T. & Stadler, H. (1978) *Eur. J. Biochem.* **90,** 479–487.
6. Stadler, H. & Tashiro, T. (1979) *Eur. J. Biochem.* **101,** 171–178.
7. Wagner, J. A. & Kelly, R. B. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4126–4130.
8. Walker, J. H., Obrocki, J. & Zimmermann, C. W. (1983) *J. Neurochem.* **41,** 209–216.
9. Carlson, S. S. & Kelly, R. B. (1983) *J. Biol. Chem.* **258,** 11082–11091.
10. DeCamilli, P., Cameron, R. & Greengard, P. (1983) *J. Cell Biol.* **96,** 1337–1354.
11. DeCamilli, P., Harris, S. M., Huttner, W. B. & Greengard, P. (1983) *J. Cell Biol.* **96,** 1355–1373.
12. Huttner, W. B., Schiebler, W., Greengard, P. & DeCamilli, P. (1983) *J. Cell Biol.* **96,** 1374–1388.
13. Ueda, T. & Greengard, P. (1977) *J. Biol. Chem.* **252,** 5155–5163.
14. Matthew, W. D., Tsavaler, L. & Reichardt, L. F. (1981) *J. Cell Biol.* **91,** 257–269.
15. Navone, F., Greengard, P. & DeCamilli, P. (1984) *Science* **226,** 1209–1211.
16. Nagy, A., Baker, R. R., Morris, S. J. & Whittaker, V. P. (1976) *Brain Res.* **109,** 285–309.
17. Higgins, R. C. & Dahmus, M. E. (1979) *Anal. Biochem.* **93,** 257–260.
18. Hager, C. A. & Burgess, R. R. (1980) *Anal. Biochem.* **109,** 76–86.
19. Köhler, G. & Milstein, C. (1975) *Nature (London)* **256,** 495–497.
20. Fazekas, de St. Groth, S. & Scheidegger, D. (1980) *J. Immunol. Methods* **35,** 1–21.
21. Goding, J. W. (1983) *Monoclonal Antibodies: Principle and Practice* (Academic, New York).
22. Hoogenraad, N., Helman, T. & Hoogenraad, J. (1983) *J. Immunol. Methods* **61,** 317–320.
23. O'Farrell, P. Z., Goodman, H. M. S. & O'Farrell, P. H. (1977) *Cell* **12,** 1133–1142.
24. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
25. Merril, C. R., Goldmann, D., Sedmann, S. A. & Ebert, M. H. (1981) *Science* **211,** 1437–1438.
26. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
27. Jahn, R., Schiebler, W. & Greengard, P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1684–1687.
28. Batteiger, B., Newhall, W. J. & Jones, R. B. (1982) *J. Immunol. Methods* **55,** 297–307.
29. Hawkes, R., Niday, E. & Gordon, J. (1982) *Anal. Biochem.* **119,** 142–147.
30. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254.
31. Markwell, M. A. K., Haas, S. M., Bieber, L. L. & Tolbert, N. E. (1978) *Anal. Biochem.* **87,** 206–210.
32. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
33. Bordier, C. (1981) *J. Biol. Chem.* **256,** 1604–1607.
34. Mugnaini, E. & Dahl, A. L. (1983) *J. Histochem. Cytochem.* **31,** 1435–1438.
35. Renfroe, J. B., Chronister, R. B., Haycock, J. W. & Waymire, J. C. (1984) *Brain Res. Bull.* **13,** 109–126.
36. Rye, D. B., Saper, C. B. & Wainer, B. H. (1984) *J. Histochem. Cytochem.* **32,** 1145–1153.
37. Nagy, A., Varady, G., Rakonczay, Z. & Plic, A. (1977) *J. Neurochem.* **29,** 449–459.
38. Buckley, K. M. & Kelly, R. B. (1984) *Soc. Neurosci. Abstr.* **10,** 919.
39. March, P. E. & Thornton, E. R. (1983) *Biochem. Biophys. Res. Commun.* **110,** 804–810.
40. Zisapel, N. & Zurgil, N. (1979) *Brain Res.* **178,** 297–310.
41. Zisapel, N. (1982) *Biochim. Biophys. Acta* **707,** 243–251.
42. Zanetta, J. P., Reeber, A. & Vincendon, G. (1981) *Biochim. Biophys. Acta* **670,** 393–400.
43. Smith, A. P. & Loh, H. H. (1981) *J. Neurochem.* **36,** 1749–1757.
44. Nagy, A. & Bock, E. (1980) *Biochim. Biophys. Acta* **600,** 103–107.