Identification of a Transmembrane Glycoprotein Specific for Secretory Vesicles of Neural and Endocrine Cells

KATHLEEN BUCKLEY and REGIS B. KELLY

Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143

ABSTRACT Several types of cells store proteins in secretory vesicles from which they are released by an appropriate stimulus. It might be expected that the secretory vesicles in different cell types use similar molecular machinery. Here we describe a transmembrane glycoprotein (Mr ~100,000) that is present in secretory vesicles in all neurons and endocrine cells studied, in species from elasmobranch fish to mammals, and in neural and endocrine cell lines. It was detected by cross-reactivity with monoclonal antibodies raised to highly purified cholinergic synaptic vesicles from the electric organ of fish. By immunoprecipitation of intact synaptic vesicles and electron microscopic immunoperoxidase labeling, we have shown that the antigenic determinant is on the cytoplasmic face of the synaptic vesicles. However, the electrophoretic mobility of the antigen synthesized in the presence of tunicamycin is reduced to Mr ~62,000, which suggests that the antigen is glycosylated and must therefore span the vesicle membrane.

It now appears likely that protein secretion by cells can take two forms (14, 16, 38). Constitutive secretion, in which the rate of protein secretion is unregulated and quite closely follows the rate of protein synthesis, occurs in almost all cell types, including liver cells, lymphocytes, and yeast cells. In contrast, the rate of secretion is highly regulated in endocrine, exocrine, and neuronal cells which respond to external signals by releasing a large amount of protein in a short period of time. Cells with regulated secretion store their secretory proteins in storage granules, whose probability of fusing with the plasma membrane is dramatically enhanced by the presence of a chemical or electrical signal. Some cells appear to have both constitutive and regulated pathways (14, 16). Cells that have the regulated secretory pathway must express gene products unique to this pathway, and proteins necessary for storage of secretory proteins, for calcium-dependent exocytosis, and for sorting of secretory proteins between the constitutive and regulated pathway, for example. Because of differences among the exocrine, endocrine, and neuronal cells, one might also expect to find proteins specific for the secretory pathway in only one of the cell types.

One potential way of identifying proteins involved in the regulated pathway is to generate antibodies specific for the storage vesicles characteristic of that pathway. This approach has been successful with other subcellular organelles. For instance, both polyclonal and monoclonal antibodies now exist that allow the relatively unambiguous recognition of clathrin-coated membranes (27), rough endoplasmic reticulum (28, 39), and the Golgi apparatus (6, 26, 28, 37, 39). The possibility of using a similar approach to identify universal components of secretory vesicles was suggested by the observation that a polyclonal antiserum to synaptic vesicles purified from the electric organ of a marine electric ray recognized not only nerve terminals in many areas of the mammalian brain, but also endocrine cells (18). We have therefore prepared a library of monoclonal antibodies that recognize unique antigens in synaptic vesicles. By screening for an antigen shared by secretory vesicles in other cells with the regulated pathway we found that one monoclonal antibody (anti-SV2) recognizes a transmembrane glycoprotein that appears to be common to all synaptic vesicles and endocrine secretory vesicles. The glycoprotein can be detected in nerve terminals of species ranging from rat and frog to cartilaginous fish. Although exocrine cells have regulated secretion, they lack the antigen.

The antibody and the protein it recognizes may provide a marker for the appearance and disappearance of the regulated pathway in neurons and endocrine cells. Because the antigenic site is on the cytoplasmic surface, the antibody is also useful for purification by immunoabsorption of membranous organelles that contain the glycoprotein. For example, it has already permitted the isolation of a subclass of bovine brain coated vesicles that contains synaptic vesicle proteins (33).
MATERIALS AND METHODS

Nerve growth factor (NGF) was generously donated by Dr. David Shelton, University of California, San Francisco (UCSF).

Preparation of Monoclonal Antibodies: Synaptic vesicles were purified from the esmolbranch DISCOPIA ovum as described for Nactne brasiliensis by Carlson et al. (10). Spleen cells from BALB/c mice immunized with synaptic vesicles were fused with SP2/0 myeloma cells by use of polyethylene glycol 4000 according to the modifications by Fazekas de St. Groth and Sheiddegger (12) of the procedure of Kohler and Milstein (22). Supernatants from the hybridomas were screened for the presence of synaptic vesicle specific antibodies using cold-phase radioimmunoassay that compares binding of antibodies to esmolbranch synaptic vesicles and to a vesicle-free side fraction of the controlled pore glass column (8).

Immunocytochemistry: Tissues from animals perfused with 4% formaldehyde were infiltrated with phosphate-buffered saline (20 mM sodium phosphate, 150 mM sodium chloride, pH 7.4) (PBS) containing 10% sucrose, mounted in Tissue-Tek, quick-frozen with dichlorodifluoromethane, and sectioned. Sections and coverslips were washed with 1% BSA-PBS and PBS, fluorescein-conjugated goat anti-mouse IgG in 1% BSA-PBS was added to sections and coverslips, then washed with PBS, fluorescein-conjugated goat anti-mouse IgG and rhodamine-conjugated sheep anti-rabbit IgG from Cappel Laboratories (West Chester, PA), horseradish peroxidase-conjugated goat anti-mouse IgG and Tissue-Tek from Miles Laboratories Inc. (Elkhart, IN), and ultrapure urea from Schwarz/Mann (Spring Valley, NY). 10 nm gold-conjugated anti-mouse IgG was obtained from Jansen Pharmaceutica, Louis, MO). Immunocytochemistry of Lowicryl-embedded tissue was done by a modification of the procedure of Altman et al. (1) and is described in detail by Reichardt, manuscript submitted for publication. Thin sections were collected on Formvar-coated nickel grids, washed with 5% normal goat serum in PBS, and incubated with undiluted hybridoma supernatant for 1 h. After three brief rinses with 1% BSA-PBS, fluorescent-conjugated goat anti-rabbit IgG in 1% BSA-PBS was added. Sections and coverslips were washed with 1% BSA-PBS and PBS, mounted in 90% glycerol, 10% PBS, 2.5% 1,4 diazobicyclo[2,2,2]octane (20) and viewed with fluorescence filters. When horseradish peroxidase-conjugated IgG was used as a second antibody on cryostat sections, 0.1% Triton X-100 was used in the 1% BSA-PBS buffer throughout the incubations and washes. After they were rinsed with PBS, the sections were reacted with 0.5 mg/ml diaminobenzidine hydrochloride and 0.01% hydrogen peroxide in 0.05 M Tris, pH 7.6, until reaction product was visible. The sections were then dehydrated in ethanol, cleared in xylene, and mounted in Permount (Fisher Scientific Co., Fair Lawn, NJ).

AIT-20 cells were stained for adrenocorticotropic hormone (ACTH) with rabbit antiserum generated as described by Moore et al. (32) and affinity purified according to the method of Mains and Eipper (29). The cells were fixed with 3% formaldehyde, pretreated with 0.2% Triton X-100 in PBS-PBS, and treated with autoreceptor as to ACTH, followed by rhodamine-conjugated sheep anti-rabbit IgG. All rinses and antibody incubations were done in 0.25% Triton X-100 in PBS-PBS.

Immunoelectronmicroscopy of electric organ nerve terminals was done as described (5).

Immunocytochemistry of Lowicryl-embedded tissue was done by a modification of the procedure of Altman et al. (1) and is described in detail by Valentino et al. (Valentino, K. L., D. A. Crumwine, and L. F. Reichardt, manuscript submitted for publication). In short, rat brains were fixed by perfusion with 4% paraformaldehyde, 0.1% glutaraldehyde, and 2 mM CaCl2 in 0.1 M cacodylate buffer. Brains were removed and fixed overnight in the same fixative but without glutaraldehyde. Pieces of cerebellum were washed in 0.1 M cacodylate buffer and stained on ice with 2% aqueous uranyl acetate at 4°C for 1-2 h. Tissue was dehydrated in graded series of ethanol and infiltrated with Lowicryl K4M as described. Valentino, K. L., D. A. Crumwine, and L. F. Reichardt, manuscript submitted for publication. Thin sections were collected on Formvar-coated nickel grids, washed with 5% normal goat serum in PBS, and incubated with undiluted hybridoma supernatant for 20 min. After a brief rinse in PBS, 0.1% w/v goat serum-PBS, the sections were floated on a drop of undiluted goat anti-mouse IgG adsorbed to 10 nm colloidal gold for 5 min. Control sections were incubated with culture medium and goat anti-mouse IgG-colloidal gold. Grids were stained with 2% OsO4 and with 2% uranyl acetate.

Random fields from both the glomerular and molecular layers of the cerebellum were photographed at 15,000 X and printed at a further magnification of 2.5. 12 micrographs were chosen from which synapses could be clearly recognized by the presence of pre- and postsynaptic densities. Presynaptic terminals with intact plasma membranes (a total of 55 terminals) were outlined with a 0.1 mm diameter micrograph circle. On each micrograph, the total number of gold particles localized over the outlined synapses were counted. The micrographs were then Xeroxed and weighed before and after the outlined synapses were cut out. The total number of gold particles in all 12 micrographs (3,293 particles) was divided by the total weight of the micrographs. This figure represents the density of the gold particles if they are randomly distributed but does not represent background binding, since synaptic binding is included in this number. The number of gold particles localized over the outlined synaptic terminals was divided by the total weight of the synapses. An increase in the density of gold particles associated with synaptic terminals over average density represents a specific association of gold particles with presynaptic structures. Control sections incubated with culture medium and goat anti-mouse IgG-colloidal gold had <10 gold particles per micrograph.

Preparation of Tissues for Immunoblots: Synaptosomes were isolated from bovine brain by a modification of published procedures (13). The tissue was homogenized in a buffer of 0.32 M sucrose, 10 mM HEPES, and 10 mM EDTA, pH 7.4 (g brain/10 ml buffer). The pellet obtained by centrifugation of the homogenate at 20,000 g for 20 min was washed by centrifugation and resuspension in homogenization buffer. 15-ml samples of the resuspended material were centrifuged at 80,000 g for 16 h in a discontinuous Ficoll gradient consisting of 8-nl layers of 2, 8, and 16% Ficoll in homogenization buffer. The synaptosome band was collected from the interface between 8 and 16% Ficoll. Chromatographically purified myelin membrane was prepared by the method of Trifaro and Dowkind (40) were a gift of Dr. Erik Schleif; membrane fractions from rabbit and chicken brain were generously donated by Dr. John Bixby, UCSF.

Cell Culture: AtT-20/D16V cells were grown in 10% horse serum in Dulbecco's minimal essential medium containing 4.5 g/liter glucose (Dulbecco's minimum essential medium-H21) at 15% CO2. The pheochromocytoma cell line, PC12, was obtained from UCSF tissue culture facility and grown in 10% fetal calf serum and 5% horse serum in Dulbecco's minimum essential medium-H21 without NGF, except for immunofluorescence studies where 2.55 NGF was added at a concentration of 100 ng/ml; HIT cells were grown in 15% heat-inactivated horse serum and 2.5% fetal calf serum in Dulbecco's minimum essential medium-H16 (1 g/liter glucose); and GH3 cells were grown in 10% fetal calf serum in Dulbecco's minimum essential medium-H21 at 10% CO2. Superior cervical ganglion neurons, dissociated from 1-2-d-old rats, were generated and cultured by Arthur Lander (UCSF). The cells were in medium containing NGF on poly-d-lysine-coated glass coverslips which had been pretreated with bovine corneal epithelial conditioned medium as previously described (24) and fixed 24 h after plating for immunofluorescence.

Metabolic Labeling with [35S]Methionine: PC12 cells were grown to confluence without NGF in 15 cm tissue culture plates. Cells were labeled at 37°C with [35S]methionine in medium containing one-twentieth the normal amount of methionine. For long term labeling, cells were incubated for 5 h in [35S]methionine and extracted immediately, or chased for 1 h in normal medium and then extracted. Cells treated with tunicamycin were incubated with 5 mg/ml tunicamycin in normal medium for 2 h before labeling was done. This concentration of tunicamycin does not inhibit protein synthesis in AtT-20 cells (A. Katzen and H.-P. Moore, unpublished observations). For short term labeling (<1 h), cells were first incubated for 20 min in medium with one-twentieth normal methionine. Cells were extracted with 0.5 ml/15 cm2 plate of ice-cold lysis buffer (1% Nonidet P40, 0.4% deoxycholate, 66 mM EDTA, 10 mM Tris-HCl, pH 7.4 [35]) containing 0.3 mg/ml phenylmethylsulfonyl fluoride and 0.3 mg/ml iodoacetamide. The cell extracts were clarified by centrifugation at 12,000 g for 5 min, and the supernatants were kept for analysis.

Preparation of Monoclonal Antibody Immunoblots and Immunoprecipitation of SV2 Antigen: A 1 ml-study of 10 mg/ml rabbit antiserum irnoprecipitated with 2c deoxycholate, 0.1% SDS, 50 mM Tris-HCl, 0.4 M NaCl, pH 7.4. Immunoprecipitations were done by a modification of the procedure of Carbon

Abbreviations used in this paper: mAb, monoclonal antibody; NGF, nerve growth factor.
and Kelly (9). Samples to be precipitated were dissolved in 1% SDS, then diluted 10-fold with 0.1% BSA in immunoprecipitation buffer, and mixed with 50 μl of immunobeads/20 μg protein overnight at 4°C. Control beads were prepared with a monoclonal antibody to a glycoprotein of elasmobranch synaptic vesicles (SV1) that does not crossreact with the mammalian nervous system.

**Immunoprecipitation of Synaptic Vesicles:** Immunoprecipitation of whole and sonicated vesicles labeled by iodination with [125I]iodosulfanilic acid was done as described by Carlson and Kelly (9) with the following modifications. Aliquots of labeled vesicles were incubated overnight with two different concentrations of monoclonal supernatant (0.5 or 1.0 μl) or culture medium without antibodies at 4°C. 50 μl of a 10 mg/ml slurry of rabbit antismouse Immunobeads was then added to each sample and incubated with rotation for 3 h at 4°C. The labeled beads were spun for 10 min at 12,000 g through a pad of 12.5% Ficoll in 0.4 M NaCl, 10 mM HEPES, 1% BSA, pH 7.0, and washed three times with buffer. The pelletted beads were counted and the amount of radioactivity precipitable counts bound to Immunobeads prepared with two different concentrations of monoclonal supernatant.

**Other Techniques:** Proteins were determined by the method of Bradford (4) by use of the Bio-Rad protein assay reagent with BSA as a standard. Approximate M, in SDS PAGE was determined by preparation of a calibration curve based on the mobilities of myosin (205,000), beta-galactosidase (116,000), carbonic anhydrase (29,000), and phosphorylase B (97,400), bovine albumin (66,000), egg albumin (45,000), and carbonic anhydrase (29,000).

**RESULTS**

**Monoclonal Antibodies Specific for Electric Organ Synaptic Vesicles**

We have generated monoclonal antibodies to unique components of cholinergic synaptic vesicles. Mice were immunized with highly purified vesicles from the electric organ or the elasmobranch *Discophy ge Dyna* and hybridoma cell lines were obtained by conventional techniques. Antibodies secreted by these cell lines were tested for specific binding to purified electric organ synaptic vesicles using a solid-phase radioimmunoassay. In a panel of six monoclonal antibodies specific for synaptic vesicles by solid-phase radioimmunoassay, antibodies to one antigen, SV2, also showed wide cross-reactivity with the mammalian nervous system. Antibodies to SV2 were further screened to show that the antibodies recognized on antigenic determinant that co-purified with elasmobranch synaptic vesicle contents during permeation chromatography on controlled pore glass columns (Fig. 1). To establish the location of the SV2 antigen in synaptic vesicles purified from the electric organ, we tested hybridoma supernatant to this antigen for its ability to precipitate intact or sonicated vesicles. Antibodies to the SV2 antigen were able to precipitate both intact and sonicated vesicles, whereas antibodies to a proteoglycan-like material (SV1) that is on the inside of synaptic vesicles (9) were able to precipitate vesicle membranes only when the vesicles had been sonicated previously (Fig. 2). We saw consistent results with electron microscopy (Fig. 3) using an immunoperoxidase technique to visualize antibody binding to nerve terminals in a preparation of gently homogenized electric organ (5). When nerve terminals were incubated with antibodies to the SV2 site, little or no reaction product was found associated with intact nerve terminals. Occasionally, however, synaptic vesicles were heavily labeled with reaction product in nerve terminals that were apparently lysed during preparation (Fig. 3b). In contrast, antibodies to the SV1 site (Fig. 3a) bound to the outside of nerve terminals as we have previously described (5). This antigen, which is on the inside of synaptic vesicles (9), is accessible on the outside of nerve terminals, presumably as a result of exocytosis of vesicle membrane. Unlike the SV2 antigen, the SV1 antigen was not detectable in damaged nerve terminals, presumably because the vesicle membrane remains intact. Thus, the SV2 antigenic site is on the cytoplasmic face of synaptic vesicle membranes whereas the SV1 site is on the luminal face of the vesicle membrane.

The SV2 Antigenic Site Is a Nerve Terminal Marker in Mammalian and Amphibian Nervous Systems

Monoclonal antibodies to electric organ synaptic vesicle
antigens were screened for cross-reactivity with neural tissue of other species by indirect immunofluorescence on cryostat sections of formaldehyde-fixed rat spinal cord. Only the monoclonal antibody to the SV2 antigen showed detectable binding. The pattern of binding seen with this antibody suggests that it is not restricted to any one class of nerve terminals but rather is a universal marker for vertebrate nerve terminals. Small, punctate fluorescence was seen in all regions of the rat and frog spinal cord. Label was particularly heavy in the substantia gelatinosa of the dorsal horn, and, in the ventral horn of the spinal cord, the cell bodies and dendrites of the motor neurons were outlined by fluorescent spots (Fig. 4, a and b). Little specific fluorescence was seen in the neuronal cytoplasm. The antigen was not restricted to neurons that innervate the peripheral nervous system because the monoclonal antibody to SV2 bound to both the glomerular and molecular layers of the rat cerebellum and all the synaptic layers of the hippocampus (Fig. 4, c and d). The SV2 antigen was also found in amphibian central nervous system, as monoclonal antibody to SV2 bound to the synaptic regions of the bullfrog retina, the inner and outer plexiform layers (Fig. 4, e and f), and to synapses in the frog spinal cord (data not shown).

To obtain further evidence for the association of SV2 with synaptic vesicles in the mammalian nervous system, we examined the binding of antibodies to SV2 to thin sections of Lowicryl-embedded rat cerebellum and visualized the binding sites with goat anti-mouse IgG adsorbed to colloidal gold. Gold particles were concentrated over presynaptic elements, which could be easily identified by the darkly stained pre- and postsynaptic densities (Fig. 5, a–c), and also over varicosities (Fig. 5a). We found few gold particles over mitochondria within nerve terminals (Fig. 5, b and c), which suggests that the antigen is associated predominantly with synaptic vesicles. To obtain some quantitative measure of the synaptic specificity of antibody binding in the cerebellum, we compared the density of gold particles over synaptic terminals to the density of the total number of gold particles in a given field, as described in Materials and Methods. We chose this method of comparison because the preservation of ultrastructural detail was variable throughout the section. Consequently, the binding sites of all the gold particles could not be assigned unambiguously to synaptic or nonsynaptic structures. The density of gold particles located over presynaptic terminals was 4.2 times greater than the average density of gold particles. Furthermore, control sections incubated with culture medium alone had <4% of the total number of gold particles seen with antibodies to SV2.

Cross-reaction with Other Secretory Cells

Because cells with the regulated secretory pathway are similar in many ways, we asked if the SV2 antigen was also present in secretory vesicles of non-neural secretory cells. The antibody showed specific binding to cells in several types of endocrine tissues in the rat, including the adrenal, pituitary, and pancreas. In the adrenal gland, the antibody bound to the endocrine cells of the medulla but not to the steroid-
producing cells of the cortex, presumably because they lack secretory vesicles (Fig. 6c). It also bound strongly to cells of the intermediate lobe of the pituitary and less strongly, but detectably, to the cells of the anterior lobe (Fig. 6, a and b).

The pancreas is composed of two types of secretory cells. The acinar cells, classified as exocrine, surround small groups of islet cells which are considered endocrine. In sections of pancreas, only the islet cells (clearly distinguishable in phase microscopy by the absence of the phase dense granules present in the acinar cells [Fig. 6f]) showed fluorescent staining after incubation with the monoclonal antibody to SV2, whereas the acinar cells had no detectable fluorescence (Fig. 6, d and e). It would seem therefore that the SV2 antigen is not a universal marker for cells with regulated secretion but is specific for neurons and endocrine cells. Other secretory tissues that did not contain detectable amounts of the SV2 antigen were rat submaxillary salivary gland, thyroid follicular cells, liver, and lymph node (data not shown).
Expression of the SV Antigen in Cultured Cells

To study the biogenesis and function of the SV2 antigen it would be an obvious advantage if the antigen were expressed in neural and endocrine cells in culture. Several cell types were tested and found to have the SV2 antigen. Neurons dissociated from superior cervical ganglia of neonatal rats were plated into NGF and fixed 24 h later during the period of maximal neurite outgrowth. Specific immunofluorescence was seen in some, but not all, of the growth cones (Fig. 7 d). Furthermore, a perinuclear staining could be seen in the neuronal cells which may represent an accumulation of antigen in the Golgi region (Fig. 7 b).

The SV2 antigen was also present in established cell lines. The pheochromocytoma cell line, PC12, was grown for a week in the presence of NGF. After incubation with monoclonal antibodies (mAb) to SV2, there was strong fluorescence of the varicosities in these cultures and in the perinuclear region of the cell cytoplasm (Fig. 8 a). Three endocrine cell lines also stained with mAb to SV2. AtT-20 cells, derived from mouse anterior pituitary, synthesize, store, and secrete the hormone ACTH (15, 34). These cells bound antibody to SV2 specifically at the tips of the short processes, where secretory vesicles are known to accumulate (21) and in the perinuclear cytoplasm, perhaps associated with the Golgi apparatus (Fig. 8 c). This pattern of staining was similar to the pattern seen when AtT-20 cells were incubated with an affinity-purified antiserum to ACTH (Fig. 8 d). GH3 cells, also derived from the anterior pituitary, which secrete growth hormone and prolactin, and HIT cells, an insulin-secreting cell line, both contained the antigen by immunofluorescence techniques (Fig. 8, b and e).

Identification of SV2 as a Glycoprotein

In the electric fish synaptic vesicles, the SV2 antigen had the broad electrophoretic mobility often associated with a glycosylated protein (Fig. 9, lane c). To determine the biochemical characteristics of the mammalian SV2 antigen, we compared the mobility of the SV2 antigen from a variety of tissues in SDS PAGE by Western blotting techniques. Fig. 9 shows that in all samples, the SV2 antigen migrates as a similarly broad band. The predominant species in the mammalian and avian brains had Mr's of ~75,000 to 85,000 (Fig. 9, lanes d-g). SV2 antigen from endocrine cells appeared to have a higher molecular weight range of ~105,000-110,000, regardless of the species (Fig. 9, lanes h-j). The Mr of the SV2 antigen in electric fish brain (Fig. 9, lane a) and electromotor nucleus (Fig. 9, lane b) was slightly lower than the Mr of SV2 antigen in purified synaptic vesicles (Fig. 9, lane c). To determine if the wide range of apparent molecular weight could arise because the SV2 antigen is highly glycosylated, we grew PC12 cells in the presence of tunicamycin, which prevents the addition of N-linked sugars (19), and [35S]methionine. After tunicamycin treatment, the antigen immunoprecipitated by mAb to SV2 appeared as a single band at ~62,000 Mr (Fig. 10, lower arrowhead, lane c), in contrast to the antigen from untreated cells, which ran at ~110,000 (Fig. 10, arrow, lane b). Both were absent in the control (lane d).

The identity of the 62,000 Mr protein as a nonglycosylated or precursor form of the SV2 antigen was supported by comparison of the mobility of labeled material extracted from cells immediately after a 30-45 min pulse of [35S]methionine to material from cells that were labeled for 5 h and then chased for an hour in medium without [35S]methionine. Fig. 10 shows that after short term labeling the SV2 antigen appeared as a single band at lower molecular weight (~64,000, lane a), whereas the higher molecular weight form appeared (lane b) after much longer labeling.

DISCUSSION

The similarities between the calcium-regulated exocytotic events in neuronal, endocrine, and exocrine cells suggests that some of the molecular machinery involved in regulated release might be shared. If the shared molecules are highly conserved then there should be common antigenic determinants in all cells that have the regulated pathway. In searching for such a determinant, we have identified a transmembrane glycoprotein present in highly purified synaptic vesicles from elaso-
FIGURE 6 Binding to endocrine cells of antibody to the SV2 antigen. (a) Immunofluorescence staining of the rat intermediate pituitary with monoclonal antibody to SV2. (b) Immunofluorescence of the rat anterior pituitary. (c) Immunoperoxidase staining of the adrenal medulla (m). The cortical cells (cor) are unstained. (d and e) Immunofluorescence staining of rat pancreas. Only the islet cells are fluorescent after incubation with antibody to SV2. (f) Phase picture corresponding to fluorescent section shown in e. Note that the cells that are not fluorescent in e contain many phase dense granules, a characteristic of the exocrine cells in the pancreas. Bars: (a and b) 25 μm; (c) 50 μm; (d–f) 25 μm. (a and b) × 560. (c) × 300. (d–f) × 800.

branch electric organ that, by immunofluorescence and immunoblotting techniques, was also present in a wide variety of nerve terminals in the central and peripheral nervous system and in several types of endocrine, but not exocrine, cells.

We believe for several reasons that the antigen recognized by the anti-SV2 monoclonal antibody is glycosylated. First, the mobility of the protein in a variety of tissues and species in SDS PAGE was heterogeneous (Fig. 9). Second, when PC12 cells were grown in the presence of tunicamycin, a drug that
blocks the addition of asparagine-linked sugars, the apparent
molecular weight of the protein in SDS PAGE was reduced
by ~30,000 (Fig. 10). Furthermore, the short and long term
labeling experiments showed that the protein migrated as a
single band with an $M_r$ of 64,000 after short term labeling
and appeared as a higher molecular weight form after longer
labeling periods (Fig. 10, lanes a and b). The slightly higher
molecular weight of this precursor form of the SV2 protein
as compared with the molecular weight of the protein synthe-
sized in the presence of tunicamycin (Fig. 10, lane c) is
probably due to co-translational glycosylation of the precursor
protein (19).

Because glycosylation of most, and probably all, proteins
occurs on the luminal side of the membrane, and the anti-
odies to SV2 bound to the cytoplasmic side of the vesicle
membrane (Figs. 2 and 3), we assume that the SV2 glycopro-
tein spans the vesicle membrane. Furthermore, the antigen is
recovered in the detergent phase after solubilization of bovine
brain synaptosomes with Triton X-114 (C. K. Kassenbrock,
unpublished observations), as is characteristic of integral
membrane proteins (3). It should therefore also be possible to
generate antibodies to the luminal domain. Such antibodies
would be of great value in determining the fate of the glyco-
protein after it is externalized by exocytosis. They also would
facilitate the selection of variant strains of cells that lack the
protein.

Comparison of the mobilities of the SV2 glycoprotein from
endocrine and neural sources suggests that the protein in
endocrine secretory granules differs slightly from that in neu-
ronal synaptic vesicles. Although there was some variation in
the SV2 antigen in brain homogenates from different species,
the differences between the electrophoretic mobility in bovine,
chicken, mouse, and rabbit brain were slight as compared
with the difference between the average of the brain samples
and that for three endocrine cell types: PC12 cells, AtT-20
cells, and bovine adrenal chromaffin cells (Fig. 9). Further-
more, a direct comparison of bovine brain (Fig. 9, lane g)
and bovine adrenal chromaffin cells (Fig. 9, lane h) demon-
strates that the difference cannot be explained by species variation alone. Alternatively, the variation in $M_r$ of the SV2 antigen may be correlated with central and peripheral nervous system. The difference in molecular weight of the SV2 antigen in different tissues and species is probably due to the degree of glycosylation of the same polypeptide chain, as is the case with NCAM, a glycoprotein involved in neuronal adhesion. This glycoprotein has different molecular weights in embryonic chick brain and retina, but removal of sialic acid with neuraminidase converts both forms of the molecule to the same molecular weight (17). The possibility has not been eliminated, however, that the apparent difference in $M_r$ of the SV2 antigen in different tissues is due not to glycosylation but to differences in the polypeptide sequence or length.

Only one other membrane protein has been found in both synaptic vesicles and endocrine secretory granules. The protein, described by Matthew et al. (31) has a molecular weight of 65,000 in SDS PAGE. The 65,000-D protein has a distribution in rat nervous system and endocrine tissue that is very similar to that described here for the SV2 glycoprotein. Unlike the SV2 antigen, however, the protein described by Matthew et al. (31) could not be detected in electric organ synaptic vesicles (30) and its molecular weight is the same in all species of brains. The two proteins also appear to be in different populations of bovine brain coated vesicles (33). Synaptic terminals throughout the central and peripheral nervous system also contain a third protein, synapsin I (11). Unlike the 65,000-D protein and the SV2 glycoprotein, synapsin I is a peripheral membrane protein and is not detectable in the adrenal medulla, which suggests that it is associated only with neuronal synaptic vesicles and not with endocrine secretory granules (11).

The absence of the SV2 antigen from exocrine cells could reflect a function unique to secretion from neural and endo-
crine cells. Alternatively, the protein and its function may be
shared by both types of secretory granules, but the antigenic
determinant may not be conserved in exocrine cells. Because
of the similarities in the mechanisms of secretion in endocrine
and exocrine cells, it has been suggested that a common
secretory stem cell gave rise to exocrine and a neural-
endocrine precursor cell. According to this hypothesis, neu-
rons and endocrine cells then differentiated from each other
(25). Several lines of evidence suggest that endocrine and
neural cells are more closely related to each other than to
exocrine cells. Both neurons and endocrine cells secrete many
of the same peptides and hormones (23, 25); at least two
secretory vesicle membrane proteins (the 65,000-D protein
and the SV2 glycoprotein) are common to both types of cells
but absent from exocrine cells (reference 31 and this paper);
and neuron-specific enolase, previously thought to be specific
for neurons, has been found in a wide variety of the endocrine
cells but not exocrine cells (2, 36). If endocrine and neuronal
cells are closely related by evolution, the absence of the SV2
antigenic site in exocrine cells could result from tissue specific
expression of the protein rather than from a basic difference
in exocytotic mechanisms.

The widespread distribution of the SV2 antigen in tissue
ranging from the elasmobranch to the mammalian brain, and
its presence in all neuronal and endocrine tissue we examined,
suggests a conserved function for the SV2 glycoprotein. The
conserved domain is on the portion of the molecule that faces
the cytoplasm. One explanation for a conserved domain on
the cytoplasmic surface of secretory vesicles is that it allows
interaction with another conserved molecule, for example, a
component of the cytoskeleton or the plasma membrane. The
existence of such interactions might be testable by microin-
jection, reconstitution, or a mutational approach.

We thank Leslie Spector for her skillful and patient preparation of
the manuscript; Steven Carlson for his extremely generous contribu-
tion of the data in Figure 2 on immunoprecipitation of synaptic
vesicles; Lois Clift-Grady for assistance with the bovine brain
synaptosomes and for generation of an endless supply of synaptic
vesicles; Deborah Crumrine for preparation of the Lowicryl sections;
and Pico Caroni and Erik Schweitzer for their participation in gen-
erating the monoclonal antibodies. We are also grateful to the mem-
bers of the Kelly lab and Jeffrey Boone Miller for critical reading of
the manuscript and many helpful suggestions. We thank David
Shelton for his generous gift of 2.5S NGF and Arthur Lander for
donating sympathetic neurons and bovine corneal epithelium con-
ditioned medium.

A preliminary account of this work was presented at the 14th
Annual Meeting of the Society for Neuroscience, Anaheim, Califor-
nia, October 1984.

Received for publication 3 October 1984, and in revised form 11
December 1984.

REFERENCES

1. Altman, L. G., B. G. Schneider, and D. S. Papermaster. 1983. Rapid (4 hr) method for
(Abstr.)

specific enolase: a common marker for the endocrine cells and innervation of the gut and


5. Buckley, K. M., E. Schweitzer, G. Miljanich, L. Cliff-O'Grady, P. Kushner, L. Reichardt,
and R. B. Kelly. 1983. A synaptic vesicle antigen is restricted to the junctional region of
the presynaptic plasma membrane. Proc Natl Acad Sci USA 80:7342-7346.

antibody against a 135K Golgi membrane protein. EMBO (Eur Mol Biol Soc) J.
1:1621-1626.

7. Burnette, W. N. 1981. "Western Blotting": electrophoretic transfer of proteins from


