REVIEW ARTICLE

The synaptic vesicle and the cytoskeleton

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INTRODUCTION

Neurones communicate with each other predominantly by secreting neurotransmitters [1–3]. The chemical nature of neurotransmitters was investigated early in this century by Loewi (1921) [4] and Dale (1937) [5] and the important role of calcium as the ‘trigger’ in neurotransmitter release was recognized some 40 years ago [6,7]. Electrophysiological studies led to the quantal theory of synaptic transmission [8] shortly before the observation of small synaptic vesicles in axon terminals by electron microscopy [9]. This led to the suggestion by Del Castillo & Katz (1956) [10] that these synaptic vesicles were the morphological equivalents of electrophysiologically observed quanta and that the transmitter was being released into the synaptic cleft by exocytosis. This idea was strongly reinforced by the isolation of synaptic vesicles from mammalian nerve terminals by Whittaker and coworkers (1964) [11] and the demonstration that they contained the neurotransmitter acetylcholine. Since this time considerable evidence has accumulated to confirm the vesicle hypothesis, i.e. that neurotransmitters are released from nerve endings by exocytosis of synaptic vesicles. Especially impressive evidence (albeit using non-physiological stimuli) comes from the quick-freezing experiments of Heuser and coworkers (1978) [12] and Ceccarelli and coworkers (1985) [13]. Essentially it has been possible to demonstrate vesicle fusion with the plasma membrane at precisely the same time as quanta are released. Further convincing evidence comes from the demonstration that stimulation of transmitter release at the neuromuscular junction exposes antigens known to be orientated exclusively on the inside of acetylcholine-containing synaptic vesicles [14]. These data, together with the biochemical and subcellular fraction studies of Whittaker and coworkers [15], provide compelling evidence for a belief in the release of acetylcholine from synaptic vesicles by exocytosis in a manner essentially identical with that proven to occur for catecholamines from chromaffin cells [16] and very likely also to be true for noradrenergic vesicles in the central nervous system [17,18]. Despite the convincing evidence for release of neurotransmitters by exocytosis, several groups have obtained interesting results which suggest that in some cases non-vesicular release of acetylcholine occurs [19,20]. It is however generally accepted that synaptic vesicles are a storage depot of transmitter, and if not for acetylcholine then for noradrenergic vesicles there is little controversy over exocytosis as the means of transmitter release. In this review we want to describe the current view of the synaptic vesicle as an important secretory organelle which is becoming increasingly understood in terms of its biochemical composition and its interaction with the cytoskeleton. This is an area in which morphological

data show a close association of synaptic vesicles with various cytoskeletal elements (Fig. 1) [21] and where, as we shall discuss, recent findings are leading to a better understanding of how synaptic vesicles interact with microtubules and with actin-based cytoskeleton.

We shall often refer to work done on two especially popular model systems, namely the cholinergic synapse of *Torpedo* electric organ and the catecholaminergic chromaffin cell of the adrenal medulla. For more details of these systems the reader is directed to the extensive reviews which exist: for *Torpedo*, Whittaker (1977) [22] and (1984) [23]; for chromaffin cell, Winkler (1977) [24], Winkler & Carmichael (1982) [25], Burgoyne (1984) [26] and Carmichael & Winkler (1985) [27].

We do not propose to deal with transmitter contents of synaptic vesicles nor with transmitter-specific proteins involved in synthesis and uptake of specific neurotransmitters. Our perspective is the vesicle membrane and its interactions with the cytoskeleton. Such interactions are probably important at all stages in the life cycle of the synaptic vesicle, including its formation in the Golgi and its transport along the axon to the site of release. Structural proteins also anchor the synaptic vesicle in the nerve terminal and are probably involved in exocytosis, the recovery of membrane and reformation of the synaptic vesicle.

ISOLATION OF SYNAPTIC VESICLES

The method originated by Whittaker and coworkers [11] for isolating synaptic vesicles from mammalian brain involves the isolation of a crude synaptosomal fraction which is then subjected to hypo-osmotic lysis to release the synaptic vesicles. These vesicles are then separated by centrifugation on a sucrose density gradient. Further purification of the synaptic vesicles can be achieved by use of gel filtration on glass-bead columns [28–30]. Ionic interactions between the vesicle membrane and the column can result in subfractionation of the synaptic vesicles into populations containing different transmitters [29]. More recently, chromatography on Sephacryl S-1000 has also been used to purify mammalian brain synaptic vesicles [32].

Synaptic vesicles containing specific transmitters have been purified from enriched sources. The most used model system is the electric organ of electric ray fish such as *Torpedo marmorata*, which contains large amounts of entirely cholinergic nerve terminals [23]. Synaptic vesicles have been isolated from this source predominantly by sucrose density gradient centrifugation in zonal rotors [33] or sucrose density gradient flotation centrifugation [34]. As with mammalian brain synaptic vesicles, chromatography on glass bead columns may also be used to isolate cholinergic synaptic vesicles from

Fig. 1. Deep-etch view of a frog neuromuscular junction

The nerve terminal contains occasional actin filaments (a) and coated vesicles (*) in addition to many synaptic vesicles. Magnification $\times 208\,000$. Reproduced from [21] with permission. Sc, synaptic cleft.

Torpedo [28,34]. A useful system for isolating mammalian cholinergic synaptic vesicles is the myenteric plexus-longitudinal muscle preparation of the guinea pig ileum [35,36], which can also be used for isolating synaptic vesicles containing neuropeptides [37]. A major problem associated with the isolation of synaptic vesicles is the low yield of protein obtained for the most highly purified vesicle preparations. Normally only around 1 mg of synaptic vesicle protein will be obtained from the brains of 10 rats (150–200 g body weight). Similarly, hundreds of grams of electric organ tissue are required to yield milligram quantities of synaptic vesicles. It is also likely that in some cases the vesicles are still contaminated with non-vesicular soluble and membrane components. Such contamination is indeed a major problem in interpreting some of the reported studies showing an association of specific proteins with synaptic vesicles. A further problem is that variation in the ionic composition of isolation buffers may also lead to differences in the polypeptides associated with synaptic vesicles.

MACROMOLECULAR COMPONENTS OF SYNAPTIC VESICLES

The protein composition of rat brain synaptic vesicles is shown schematically in Fig. 2 together with a tentative identification of the components based on the literature.

Cytoskeletal components

Synapsin I (Protein I). Synapsin I is certainly the most characterized synaptic vesicle-associated protein yet identified [31]. Synapsin I is the collective name for two almost identical polypeptides of M_r 85000 (IA) and 80000 (IB) which are prominent neuron-specific protein substrates for both cyclic AMP-dependent and Ca^{2+} -calmodulin-dependent protein kinases in the mammalian central nervous system [38,39]. The phosphorylation is stimulated in various preparations under physiological conditions [31,40] and it is agreed that the phosphorylation of synapsin I is an important process concomitant with transmitter release.

Subcellular fractionation studies demonstrated that synapsin I was concentrated in nerve terminals and under conditions of low ionic strength synapsin I was found to be associated with synaptic vesicles during their isolation [41]. An association with synaptic vesicles was also demonstrated by electron microscopy immunohistochemistry [42] with the association specific for small synaptic vesicles [43].

Synapsin I has been purified and extensively characterized in terms of its structure. It is an elongated, highly asymmetric, molecule consisting of a highly basic, collagenase-sensitive tail region and a collagenase-insensitive head region. The head region contains one serine residue that is phosphorylated by both cyclic AMP-dependent protein kinase and by Ca^{2+} -cal-

Fig. 2. A speculative cartoon of the possible constituents of synaptic vesicle membranes

Key: CG, components only known to be associated with chromaffin granules; ?, components for which the evidence of an association with synaptic vesicles is speculative; AA, arachidonic acid; PG, prostaglandins; IP₃, inositol trisphosphate; PLA₂, phospholipase A₂; PLC, phospholipase C; PI, phosphatidylinositol.

modulin-dependent protein kinase I [44]. Two serine residues in the tail region are also subject to phosphorylation by Ca²⁺-calmodulin-dependent protein kinase II, an enzyme which is itself associated with purified synaptic vesicles [45]. Phosphorylation of the tail region decreases the binding of synapsin I from synaptic vesicle membranes [41,45] which could be consistent with its removal from vesicle membranes prior to exocytosis. One problem with the association of synapsin I with the synaptic vesicle membrane is the lack of a clear demonstration of this association under physiological ionic conditions.

Recently, another interesting direction in synapsin I research has come from studies on the neuronal cytoskeleton. Purified synapsin I binds *in vitro* to spectrin ($K_d = 5 \times 10^{-7}$ M [46,153,154,156]), to neurofilaments [47] and to microtubules [48]. Synapsin I also copurifies with microtubules [47]. The association with neuronal cytoskeleton has been confirmed by immunohistochemical studies [47]. Finally it has been suggested that synapsin I is to some extent related immunologically and in several other physicochemical properties to protein 4.1 [46,49], which is an important component of the red blood cell membrane believed to cross-link glycophorin to spectrin and to actin [50]. Only limited sequence data is available for protein 4.1, whereas synapsin has been completely sequenced. As yet no sequence homology has been established with protein 4.1 [156,157] although some homology exists with the actin-binding proteins profilin and villin. These most recent studies argue strongly for a role of synapsin I in synaptic vesicle membrane interaction with the cytoskeleton. Possible roles could include movement of synaptic vesicles to the site of release and synaptic vesicle membrane retrieval after exocytosis. Interestingly, synapsin I has recently been shown to

cross-link synaptic vesicles to synaptic plasma membranes [158].

Actin and actin-binding proteins. Light microscopic studies on mammalian brain have shown that F-actin and myosin are concentrated in regions containing synaptic terminals [51]. Morphological studies on mammalian brain [52] and on the neuromuscular junction (Fig. 1) by Hirokawa & Heuser (1982) [21] at the level of resolution of the electron microscope have shown an association of actin filaments with synaptic vesicles. At present there is little biochemical evidence for an association of actin with synaptic vesicles isolated from mammalian brain, despite the fact that synapsin I is an actin-binding protein *in vitro* [31]. A variety of actin-binding proteins are known to be present in brain tissues [53,54], including the spectrin-like protein, fodrin [55,56], a villin-like protein (90 kDa), profilin and tropomyosin [57]. However, little is known about the possible association of these actin-binding proteins with the synaptic vesicle.

Purified *Torpedo* cholinergic synaptic vesicles contain actin as a major component [33] although again the nature of its interaction with the vesicle membrane has not been investigated. To date the chromaffin granule has provided the best model system for information on the association of actin with synaptic vesicle membranes. Isolated chromaffin granule membranes contain actin [58] and isolated granule membranes have been shown to interact with actin filaments by sucrose gradient centrifugation assays [59], electron microscopic assays [60] and by viscosity measurements [61]. Actin almost certainly interacts with the chromaffin granule membrane via actin-binding proteins. α -Actinin can be shown to be

Table 1. Synaptic vesicle components and those peripherally associatedAbbreviations: T, *Torpedo* cholinergic vesicles; M, mammalian synaptic vesicles; C, chromaffin granules.

Component	$10^3 \times M_r$	Remarks	Source	Reference
Cytoskeletal components				
Synapsin I	86 + 80	Phosphoprotein	M	31
Protein III	74 + 55	Related to synapsin I	M	31
Actin	42		T	33
Tubulin ($\alpha + \beta$)	50 + 50		M	76
Clathrin	180 + 36 + 33		M	81
Enzymes and regulatory proteins				
Mg ²⁺ -ATPase	51 + 50 + 28 + 10	Proton pump	M	32
Calmodulin-dependent protein kinase II	60 + (58) + 50		M	45
Protein kinase C	80		C	110
Calmodulin	18		M	112
			T	113,114
			T	115
Calcium-binding proteins	70 + 30–36	'Calelectrin'	T	123
Lipomodulin(?)	40	'Lipocortin/s'	C	110
			M	106
			C	110
Phospholipase A ₂	?		M	106
Antigenic components of unknown function				
Chromogranin A	75	M_r 48 000 + O-linked sugars	C	139
Secretogranin I	105/113		C	140
Secretogranin II	86/84		C	140
Proteoglycan			T	142
			T	143
			C	139
Synaptophysin; p38	38	Calcium binding	Widespread M	8,145,147
p65	65		Widespread M	148
SV 2	60–110	M_r 62 000 + N-linked sugars	Widespread M,T,C	149

present in chromaffin granule membranes biochemically, and immunocytochemical studies also indicate an association with the chromaffin granules [62,63]. The α -actinin was shown to be on the cytoplasmic face of the chromaffin granule by protease-digestion experiments. Interactions between chromaffin granule membranes and F-actin *in vitro* are inhibited by calcium [61]. Several calcium-dependent actin-binding proteins have been shown to be present in the adrenal gland, including α and β fodrin (240 kDa and 235 kDa) [64], gelsolin (91 kDa) [65] and caldesmon (70 kDa) [66]. Fodrin seems to be primarily located at the plasma membrane of the chromaffin cell from studies with the light microscope [64,67] and with the electron microscope [68]. Stimulation of exocytosis is accompanied by clearing of fodrin and of caldesmon from the plasma membrane, suggesting that calcium influx is involved in opening up the structure of the cortical cytoplasm to allow secretory granules to reach their sites of exocytosis. This is not likely however to be involved in the secretion of those granules which respond immediately to cell stimulation by fusing with the plasma membrane, since drugs which bind to actin have no effect on exocytosis [69]. Although primarily

associated with the plasma membrane, fodrin seems to be present in purified chromaffin granules and caldesmon is one of a group of proteins which bind to chromaffin granules in a calcium-dependent manner [152].

Tubulin and microtubule-associated proteins. Microtubules are highly concentrated in axons where they mediate the movement of membrane vesicles [70,71]. Recently studies on the giant axon of the squid have yielded vital clues to the mechanism of axonal transport, thanks to the technique of video-enhanced differential interference contrast microscopy. This method has shown the movement of organelles along single microtubules and has enabled reconstituted systems to be developed to assay for soluble factors involved in axonal transport. The transport system will move polystyrene beads and fragments of glass [72] in addition to membrane-bound organelles. Initial studies identified a heat-sensitive, ATP-dependent protein factor of M_r 110 000, kinesin [73]. Subsequently it was shown that a second protein factor mediates movement along microtubules in the opposite direction [71]. Cholinergic synaptic vesicles purified from electric rays can be

transported along squid axon microtubules [74] and there is evidence for this mechanism carrying vesicular structures into the nerve ending.

Synaptic vesicles isolated from mammalian brain are reported by some authors to contain α and β tubulin as major proteins [75,76]. However, the vesicle isolation procedures used can be severely criticized since Burke & DeLorenzo (1982) [76] relied entirely on differential centrifugation of a hypo-osmotic lysate of a crude synaptosomal fraction, and Zisapel *et al.* (1980) [75], who used sucrose density centrifugation, produced a synaptic vesicle fraction which contained no Triton X-100-soluble protein components. Nevertheless, as much as 28 % of the soluble protein of purified synaptosomes is tubulin [77] and a direct or indirect association of synaptic vesicles with microtubules is almost certain to play an important role in synaptic vesicle movement. Synapsin I has been shown to bind to microtubules [48] and may therefore provide one of the missing links in the chain which binds synaptic vesicles to the translocation mechanism. High- M_r microtubule-associated proteins have been found in synaptosomal cytosol [78] and the staining pattern of τ proteins in mammalian cerebellum is very similar to that of nerve terminal markers [42,79] which may suggest that τ proteins may also be present in the nerve terminal. Tubulin and a protein of M_r 50000 related to τ proteins have also been shown to copurify with coated vesicles from mammalian brain [80]. Both tubulin and the 50 kDa polypeptide appear to be tightly associated with the coated vesicle membrane, which is derived at least partly from synaptic vesicle membranes ([81] and see below). Thus an association of tubulin and microtubule-associated proteins with isolated synaptic vesicle membranes is at present likely. Chromaffin granule membranes also have binding sites for tubulin [82] and as isolated contain tubulin, although this may be from contaminating membranes [83].

Clathrin and clathrin-associated proteins. At the nerve terminal clathrin-coated vesicles are probably responsible for retrieving synaptic vesicle membranes after release of transmitter [84]. Immunohistochemical studies at the electron microscope level [85,86] have shown that synaptic vesicles are surrounded by clathrin (M_r 180000) and clathrin light chains (M_r 36000 and 33000) although the clathrin is not organized into recognizable coat-like structures. A subpopulation of coated vesicles (35 %) prepared from total mammalian brain contain synaptic vesicle antigens [81]. Interestingly, this subpopulation of coated vesicles can be isolated with antibodies to synaptic vesicle-specific antigens (p65 and SV2; see below) indicating that synaptic vesicle membranes may be accessible while the basket structure of the coated vesicle is intact. A further interesting observation made by Pfeffer & Kelly (1985) [81] and independently discovered by Wiedenmann and coworkers (1985) [87] was that the Triton X-100-soluble membrane components of coated vesicles contain major polypeptides of M_r 38000 and 29000. The M_r 38000 polypeptide has been independently identified by various groups and is discussed below as a specific marker for synaptic vesicle membranes. The manner in which the clathrin coat interacts with the synaptic vesicle membrane is not clear. The basic cage-like structure of the coated vesicle is formed from three-legged structures made from the M_r 180000, 36000 and 33000 polypeptides and known as triskelions [88].

Assembly of these into cage-like baskets can occur in the absence of other proteins, although assembly is promoted by polypeptides of M_r around 100000–110000, 50000 and 16500 [89]. There is also evidence that the M_r 100000–110000 polypeptides are involved in binding clathrin to the membrane of uncoated 'stripped' coated vesicles [88,90,91].

Enzymes and regulatory proteins

Mg²⁺-activated ATPase. A variety of intracellular membrane-bound organelles, including secretory granules [92], lysosomes [93] and coated vesicles [94,95], contain ATP-driven proton pumps which acidify the inner compartment of the organelle. The chromaffin granule contains a Mg²⁺-activated ATPase which in its molecular size, subunit composition and physical appearance resembles the mitochondrial F₁ ATPase, consisting of an F₁ subunit with α , β and γ chains of M_r 51000, 50000 and 28000 and an F₀ subunit with M_r 10000 [96]. Synaptic vesicles from mammalian brain have also been shown to contain a Mg²⁺-activated ATPase activity which co-purifies on density gradient centrifugation and gel filtration with specific vesicle markers including neurotransmitter [28,97]. This protein resembles the mitochondrial F₁-ATPase in many respects, but is not identical since it is not inhibited by oligomycin. It is likely that a similar protein is present in *Torpedo* cholinergic synaptic vesicles since these also contain an environment of acidic pH [98]. The proton gradient may serve to enable uptake of specific substances into the vesicle.

Protein kinases and phosphatases. Protein kinases are essential regulators of cellular function which have been extensively studied in many tissues, including brain [99,100]. Essentially two classes of protein kinase have been defined. The first class phosphorylates cellular proteins in response to the second messengers cyclic AMP, cyclic GMP and Ca²⁺. The second class are not regulated by Ca²⁺ and cyclic nucleotides. In terms of mammalian brain synaptic vesicles synapsin I, as discussed above, is an important substrate for cyclic AMP-dependent and Ca²⁺-calmodulin-dependent protein kinases. Interestingly, highly purified synaptic vesicles contain endogenous Ca²⁺-calmodulin-dependent protein kinase II (CAM kinase II) [41,45]. This kinase has subunits of M_r 50000, 60000 and 58000. All of these polypeptides bind calmodulin and are phosphorylated on the addition of [γ -³²P]ATP. Immunocytochemical localization of the kinase demonstrates that, although it is primarily associated with dendrites, positive staining is observed in nerve terminals, possibly in association with synaptic vesicles [101,102]. In addition to synapsin I, CAM kinase II phosphorylates microtubule-associated protein 2 (M_r 270000), τ protein [159] and tubulin [47]. Phosphorylation of microtubule-associated proteins regulates tubulin polymerization and could be of relevance to synaptic vesicle movement. CAM kinase II has also been found to copurify with microtubules [158]. Interestingly, CAM kinase II has recently been shown to be Ca²⁺- and calmodulin-dependent only in the non-phosphorylated form [103]. Once phosphorylated, the enzyme can remain active for prolonged periods. This could clearly be of great significance for processes such as memory where the initial signal must produce a prolonged change in signalling between neurones. A phosphatase which acts on CAM kinase II has been identified [104] but has not

yet been characterized. A Ca^{2+} -calmodulin-dependent protein kinase has also been identified in brain coated vesicles [105]. Ca^{2+} -calmodulin-dependent kinase I (M_r 42000, 31000 and 37000) and cyclic GMP-dependent kinases (M_r 74000) are soluble proteins not known to interact with synaptic vesicles. Cyclic AMP-dependent protein kinase exists in soluble and particulate forms and phosphorylates synapsin I [99]. It is not known to be associated with the synaptic vesicle membranes. However, some reports suggest that it is present as an endogenous component in the synaptic vesicle preparations [106]. Protein kinase C (Ca^{2+} -phospholipid-dependent, M_r 80000–87000) is the prime candidate for a regulator of secretion in many cell types [69,107]. Immunofluorescence staining has shown an association of protein kinase C with secretory granules of rat bone marrow granulocytes [108] and a heavy localization in nerve terminals has also been reported [109]. Protein kinase C has not yet been found associated with mammalian brain synaptic vesicles. Interestingly, chromaffin granule membranes have been found to bind protein kinase C in a Ca^{2+} -dependent manner [110].

In addition, chromaffin granule membranes contain the tyrosine-specific protein kinase pp60^{c-src} [111] which is homologous to the protein coded for by the *src* gene of Rous sarcoma virus [99]. Brain tissue contains the highest levels of pp60^{c-src} of any non-transformed tissue, but it is not yet known if this kinase is associated with synaptic vesicles.

Protein phosphatases and their regulators are not known to be associated with synaptic vesicles, but are clearly important in the regulation of synaptic function by protein phosphorylation [99].

Calmodulin. Calmodulin (M_r 18000) is an important regulator of nerve terminal function as discussed above. Mammalian brain synaptic vesicles have been reported to contain and to bind calmodulin, as have coated vesicles [112] although apparently less than one molecule of calmodulin is bound per coated vesicle [91]. Cholinergic synaptic vesicles from electric rays have been shown to contain calmodulin in a Ca^{2+} -dependent manner [113] and also calmodulin-binding proteins [114,115].

Interestingly, Hooper & Kelly (1984) [114] found little difference between synaptic vesicles and total synaptosomes in terms of the calmodulin-binding proteins, whereas Walker *et al.* (1984) [115] found a specific enrichment of calmodulin-binding proteins of M_r 160000, 25000 and 20000. The components of M_r 25000 and 20000 corresponded to major Coomassie-stained bands of the cholinergic vesicle preparation. Calmodulin has also been shown to stimulate $^{45}\text{Ca}^{2+}$ uptake into cholinergic synaptic vesicles and to be regulated by a Ca^{2+} -calmodulin-dependent protein kinase present in the synaptic vesicle preparation [116]. In this study, Ca^{2+} resulted in the phosphorylation of polypeptides of M_r 64000, 58000, 54000 and 41000.

Ca^{2+} -dependent membrane-binding proteins. Several groups have independently identified a group of proteins (M_r 70000 and 30000–35000) which are associated with membrane cytoskeletons in a Ca^{2+} -dependent manner [117,118]. These proteins have not been extensively investigated in mammalian brain, and although they are

present [119,120,121] no studies have yet been performed on their possible localization at the synapse.

In cholinergic synapses from *Torpedo* a protein immunologically related to these proteins has been identified and called 'calelectrin' [122]. Immunohistochemical staining at the level of resolution of the electron microscope showed a possible association of calelectrin with synaptic vesicles [123] although the most prominent location of this protein was in the postsynaptic cell on the cytoplasmic face of the acetylcholine-receptor-enriched membrane. Calelectrin was also strongly associated with mitochondrial outer membrane and with filamentous structure in the postsynaptic cell.

Proteins have also been isolated from chromaffin cells which can bind to chromaffin granule membranes in a Ca^{2+} -dependent manner [124,110]. The major proteins observed had M_r 70000 and 30000–35000 and on two-dimensional electrophoresis produced a complex pattern of protein components [110,125]. Interestingly, this group of proteins includes protein kinase C [110] and a phosphatidylinositol-specific phospholipase C [126]. In addition, a major granule binding protein is p36, a protein substrate for the tyrosine kinase pp60^{c-src} [125,127] which has been shown to be a component of the chromaffin granule membrane [111]. p36 (reviewed in [118]) was originally described in cells transformed by the oncogene pp60^{v-src}. Subsequently it was identified as a major component of the cytoskeleton of intestinal enterocyte brush borders, where it exists in close association with an 11 kDa polypeptide related to the Ca^{2+} -binding protein S100 [160,161]. p36 binds spectrin and actin and its interaction with actin is regulated by phosphorylation [162]. p36 is one of two lipid-binding and F-actin-binding proteins known as lipocortins, or calpactins [128], which have an inhibitory activity against phospholipase A_2 [129]. This inhibitory activity can be switched off by phosphorylation of p36 on serine residues. If these proteins are normally associated with chromaffin granule membranes in the cell then they could regulate events including phosphorylation of proteins and the hydrolysis of phospholipids to produce diacylglycerol, inositol phosphates, lysophospholipids and arachidonic acid. Chromaffin granules are enriched in lysophospholipids [25] and arachidonic acid can induce fusion of chromaffin granule membranes [130]. Lysophospholipids are also able to induce membrane fusion [131].

One great problem with the work on chromaffin granule binding proteins is that, although these proteins can bind to the granule membrane, it is not clear if they are normally associated with these membranes in the cell. Indeed p36 [128] and caldesmon [132] seem to be predominantly associated with the plasma membrane. Thus, although a role for these proteins in secretion is appealing, more evidence is required before they can be accepted as components of secretory granules.

Phospholipases, phosphatidylinositol kinase and the lipid composition of synaptic vesicles. The lipid compositions of synaptic vesicles from mammalian brain and *Torpedo* electric organ are given in Table 2 along with those of coated vesicles and chromaffin granules. The most interesting result is that lysophosphatidylcholine is present in chromaffin granule membranes at much higher levels than those found in synaptic vesicles. Lysophospholipids are produced by the action of phospholipase A_2 . Creutz *et al.* (1985) [126] were unable to detect a

Table 2. Lipid composition of synaptic vesicles

Lipid	Composition (% by wt. of total)			
	Synaptic vesicles			
	Mammalian brain [28]	Torpedo [23]	Chromaffin granules [25]	Coated vesicles [151]
Phosphatidylcholine	42.2	46.6	27.5	42
Phosphatidylethanolamine	36.3	29.5	31.8	33
Phosphatidylserine	11.8	12.6	2.5	4
Phosphatidylinositol	2.9	5.1	8.2	10
Sphingomyelin	4.9	5.1	12.8	8
Phosphatidic acid	1.8	0.6	1.0	—
Lysophosphatidylcholine	1.5	0.4	16.8	—

phospholipase A_2 activity in chromaffin granules or in proteins binding to chromaffin granule membranes in a Ca^{2+} -dependent manner. They did, however, find a phosphatidylinositol-specific phospholipase C, which could perhaps be implicated in the activation of secretion by protein kinase C [133]. It is perhaps also important to note that the proteins which bind to chromaffin granules in a Ca^{2+} -dependent manner include proteins shown to have phospholipase A_2 inhibitory activity (see the preceding section) which might therefore mask endogenous activity. Chromaffin granule membranes are also known to contain phosphatidylinositol kinase [25], which also implicates the phosphoinositol cycle in secretion in this system.

Mammalian brain synaptic vesicles are reported to contain phospholipase A_2 [134,135] whose activity is stimulated by calmodulin, cyclic AMP, ATP and prostaglandin $F_{2\alpha}$ and inhibited by prostaglandin E_2 . Synaptic vesicles were also found to contain a phosphoprotein of M_r 40000 which the authors suggested could be lipomodulin (lipocortin), an inhibitor of phospholipase A_2 which has been found previously in synaptic plasma membranes [136] and which has been described in the preceding section. Although this idea is interesting, lipocortins I and II, the major intracellular phospholipase inhibitors described by Huang *et al.* (1986) [129], are plasma-membrane-associated proteins not found in brain tissue.

Phospholipase A_2 activity is reported to be absent from cholinergic synaptic vesicles isolated from *Torpedo* [137].

Antigenic components of unknown function

Recently several antigenic components of synaptic vesicles have been described, thanks largely to the use of monoclonal antibodies. Although the functions of these antigens are not yet known their specific antibodies provide useful tools for studying vesicles. They also must represent important aspects of vesicle function since they are usually specific to secretory granules, conserved during evolution and often represent major components of the synaptic vesicle.

Chromogranin, secretogranin and proteoglycans. Chromaffin granules have been known for some time to contain acidic glycoproteins called chromogranins [25]. Chromogranin has an apparent M_r of 75000 on SDS/

polyacrylamide-gel electrophoresis. The M_r of the non-glycosylated protein, which has recently been cloned and sequenced, is 48000 [138]. Chromogranin A is also found in the parathyroid, pituitary and brain and a related protein is co-secreted with insulin from insulinoma cells. Chromogranin A is an *O*-glycosylated protein which is also modified post-translationally by phosphorylation on serine residues and sulphation on tyrosine residues [139]. A suggested function of chromogranin A is that it might bind Ca^{2+} which is known to be stored in various secretory granules. Other similar sulphated glycoproteins present in chromaffin granules and in a variety of other secretory granules are secretogranin I (113–115 kDa) and secretogranin II (86–84 kDa) [140] which have been suggested as possibly having a role in the packaging of regulatory peptides. The relationship of these proteins to chromogranin B (100 kDa, pI 5.2), which is also a sulphated glycoprotein, is not clear. Another sulphated component of chromaffin granules is a proteoglycan (86–100 kDa, pI 4.3–5.0) [139]. Proteoglycans seem to be components of many endocrine and transmitter-containing granules [141] and cholinergic synaptic vesicles from *Torpedo* have also been shown to contain a heparan sulphate-containing proteoglycan [142,143]. Several monoclonal antibodies have been prepared to this proteoglycan which seems to be restricted to the electromotor neurones in *Torpedo* (144). The function of this proteoglycan in *Torpedo* is not known.

Synaptophysin (p38). A synaptic vesicle component of M_r 38000 has been demonstrated independently by three groups. The protein has been called synaptophysin by Wiedenmann & Franke [87] and is referred to as p38 by the group of Greengard [145]. The highly purified synaptic vesicle fractions of Huttner *et al.* (1983) [30] clearly contain a major M_r 38000 polypeptide. This protein has been isolated by two groups [87,145] who showed that the protein is an integral membrane protein with a domain exposed on the cytoplasmic side of the vesicle. As mentioned, Pfeffer & Kelly (1985) [81] were able to immunoprecipitate a subpopulation of coated vesicles with antibodies specific to synaptic vesicles (p65 and SV2; see below). These coated vesicles contained Triton X-100-soluble proteins of M_r 38000 and 29000 which were not present in the coated vesicles which were not precipitated. Wiedenmann *et al.* (1985) [87] were also able to show that coated vesicles contained M_r 38000,

29000, 24000 and 10000 membrane-associated proteins. Monoclonal antibodies to the M_r 38000 component (now commercially available through Boehringer Mannheim) were used to demonstrate the association of this protein with synaptic vesicles by electron microscopy [146]. Synaptophysin is an acidic *N*-glycosylated protein which exists as a tetramer, spans the vesicle membrane and has a cytoplasmic domain which binds Ca^{2+} [147]. The binding of Ca^{2+} may be relevant to the function of the protein, which is reported to be the major Ca^{2+} -binding protein of the synaptic vesicle.

p65. Highly purified synaptic vesicles contain a major M_r -65000 polypeptide [30,145]. Monoclonal antibodies have been prepared which recognize this component [148]. The protein has a cytoplasmic domain which can be recognized by monoclonal antibodies even in synaptic vesicle membranes surrounded by clathrin coats [81]. The protein is highly conserved during evolution and seems to be a component of all synaptic vesicles and also is present on chromaffin granule membranes and other endocrine secretory granule membranes.

SV 2. Monoclonal antibodies specific to cholinergic synaptic vesicles defined four different antigens, SV 1–SV 4 [144]. Of these the best-characterized and most widespread is SV 2 [149]. SV 2 is a transmembrane glycoprotein containing *N*-linked sugars. The non-glycosylated protein has an M_r of 62000. When glycosylated, various M_r values are obtained for the antigen depending on the species. The M_r values observed in immunoblotting experiments range from 60000 to 110000. SV 2 has a cytoplasmic domain which, like that of p65, can be recognized by antibodies even in a subpopulation of coated vesicles. As with p65, SV 2 is also expressed in endocrine cells, including the endocrine pancreas. An antigen similar to SV 2 has also been described by Walker *et al.* (1986) [150].

SUMMARY AND THE FUTURE

Synaptic vesicles are secretory organelles of fundamental importance to the function of nervous tissue. Recently, many vesicle components have been identified and partially characterized. We now have an idea of the major protein components of the synaptic vesicle and of the proteins which might interact with the vesicle to mediate its movement to the site of release and its retrieval after exocytosis. Those vesicle components which have cytoplasmic domains seem good candidates for interaction with cytoskeletal elements and clathrin coats. The fact that these components are often also associated with secretory granules from endocrine tissues suggests that studies on vesicle components and their interactions with the cytoskeleton and with the plasma membrane might lead to a greater understanding of the process of secretion in general.

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