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MINI-REVIEW

Neurotransmitter release through the V0 sector of V-ATPase

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Neurotransmitter release occurs at specialized areas of the nerve terminal membrane, the active zones, where clusters of synaptic vesicles, the neurotransmitter-storing organelles, are observed (Couteaux and Pécot-Dechavassine 1974; Harlow *et al*. 2001). In resting conditions, a population of synaptic vesicles is docked to the active zone membrane, close to voltage-gated calcium channels (Robitaille *et al*. 1990), within microdomains where, upon stimulation, cytosolic calcium reaches transiently a very high concentration (Llinas *et al*. 1992). In spite of the high specialization of the active zone structure and high speed of synaptic transmission, proteins involved in docking and fusion of synaptic vesicles are similar to those operating for much slower membrane fusions, from yeast to neurones (Wickner and Haas 2000). In this respect, the role of SNARE complexes for docking synaptic vesicles at the active zones has been well documented (Rothman 1994; Jahn and Südhof 1999).

A detailed genetic and pharmacological dissection of yeast homotypic vacuole fusion revealed the existence, after vacuole docking by *trans*-SNARE complex formation, of a Ca^{2+}/calmodulin reaction preceeding the final microcystin-inhibited step of membrane fusion (Wickner and Haas 2000). Recently, Peters *et al*. (2001) showed that it was the proteolipids of the membrane sector (V0) of V-ATPase which bind to calmodulin and initiate the final step of membrane fusion. The vacuolar-type H^+-ATPase is indeed composed of a proteolipid membrane sector (V0) and a catalytic sector (V1). The association between V0 and V1 is reversible and participates in the regulation of proton pumping (Nelson and Harvey 1999). Reconstituted V0 proteolipids form a pore that opens in the presence of calcium and calmodulin. During the fusion of two yeast vacuoles, a V0 *trans*-complex is formed by the apposition of two proteolipid rings, brought into close contact by the SNARE proteins. The V0 *trans*-complex may therefore form a proteolipid channel spanning the two interacting membranes at the fusion site (Peters *et al*. 2001). We would like to discuss the relevance of this model for neurotransmitter release.

Synaptosomal membranes were shown to contain a proteolipid oligomer that supported a calcium-dependent release of acetylcholine (ACh) when reconstituted in artificial membranes (Israël *et al*. 1986; see Fig. 1). This oligomer (mediatophore) turned out to be made of the proteolipid c subunit of V-ATPase (Birman *et al*. 1990). When cells were transfected for this proteolipid, they acquired a Ca^+-dependent ACh release mechanism that displayed quantal properties (Falk-Vairant *et al*. 1996; see Fig. 2). Such reconstitution experiments, using liposomes, transfected cells or *Xenopus* oocytes (Cavalli *et al*. 1993), showed that a single proteolipid ring not only opens upon calcium action but is sufficient to let ACh out down its concentration gradient. This was confirmed by Peters *et al*. (2001) who measured the release of choline through reconstituted yeast V-ATPase proteolipids, release that required Ca^{2+} and, in this case, calmodulin.

In synapses, the neurotransmitter is pre-concentrated in synaptic vesicles. This process depends on the proton gradient generated by the V-ATPase, and is blocked by N-N′-dicyclohexylcarbodiimide (DCCD). In contrast, the efflux of ACh from already loaded synaptic vesicles is not affected by DCCD (Dolezal *et al*. 1993). This illustrates that ACh and protons follow different routes. Protons bind to a glutamic residue facing the exterior of the proteolipid ring (Harrison *et al*. 2000) and are translocated during the ATP-driven rotation of this ring (see Nelson and Harvey 1999 for a review on V-ATPases). ACh is most probably released through a pore found in the middle of the proteolipid oligomer by Jones *et al*. (1995).

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Abbreviations: ACh, acetylcholine; DCCD, *N-N′*-dicyclohexylcarbodiimide.

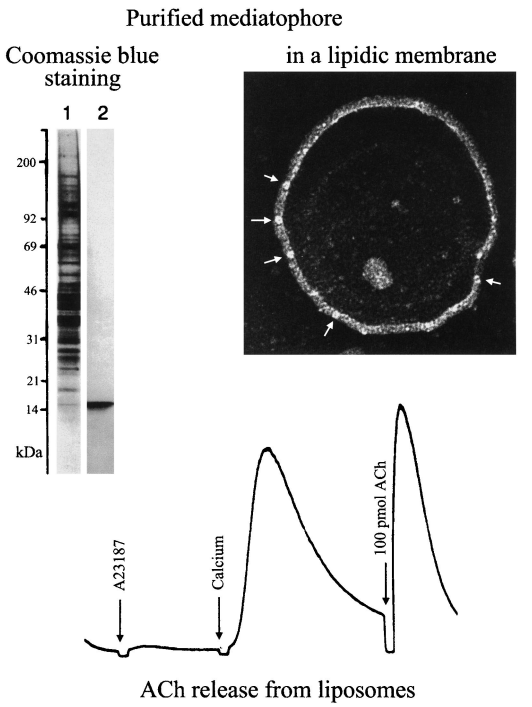

Fig. 1 SDS-gels (a) comparing total synaptosomal proteins (lane 1) and purified proteolipid V0 (mediatophore) subunit (lane 2). (b) Purified mediatophore (arrows) in its lipidic membrane environment. (c) Calcium-dependent acetylcholine (ACh) release through purified mediatophore reconstituted in proteoliposomes.

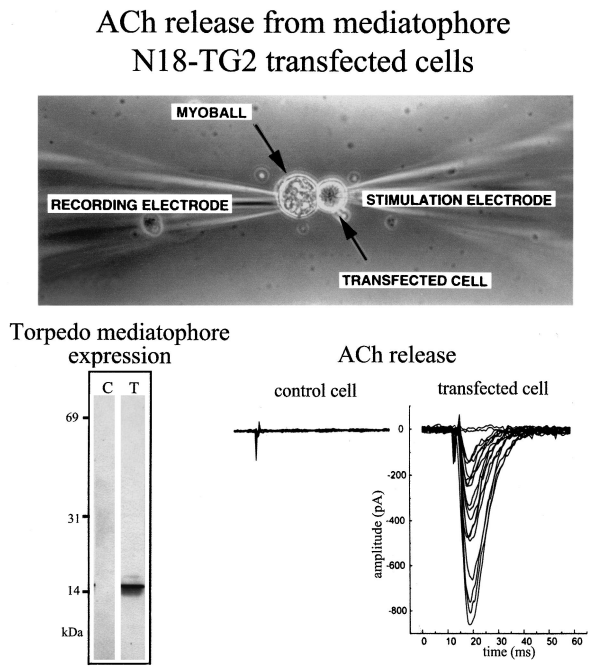

Fig. 2 Transfection of N18-TG2 neuroblastoma cells with a plasmid encoding for Torpedo mediatophore. The western blot shows that the transfected cells (T) expressed in their membranes the Torpedo proteolipid (detected with monoclonal antibody 15K1; C: control cells). Acetylcholine release from stimulated N18-TG2 cells, loaded with acetylcholine, was detected using a patch-clamped *Xenopus* myocyte (upper panel). Traces compare a control cell which does not release transmitter and a transfected cell that displays release with clear quantal jumps.

At the active zone, in addition to synaptic vesicles (Yamagata and Parsons 1989), the proteolipid rings are present in the pre-synaptic membrane. Using the fracture-label technique (Taubenblatt *et al.* 1999), we observed the presence of proteolipid subunits of V-ATPase in the plasmalemma, particularly at the site of contact between docked synaptic vesicles and the synaptosomal membrane. Strickingly, the V0 proteolipid (mediatophore) in the pre-synaptic membrane is associated with large intramembrane particles (Fig. 3). The number of such large intramembrane particles abruptly increases at the peak of ACh release (Muller *et al.* 1987), when the fusion pore is supposed to open. Similar particles also increase in number during release in cells transfected with the V0 proteolipid (Bugnard *et al.* 1999). In addition, large intramembrane particles are stably present in the active zone membrane, organized in relation with docked synaptic vesicles (Heuser *et al.* 1979; Harlow *et al.* 2001). Besides pre-synaptic Ca^{2+} channels, the large V0 *trans*-complex identified by Peters *et al.* (2001) could well be constituents of a population of active zone intramembrane particles.

The two proteolipid rings, i.e. the vesicular V0 and the membrane V0 (mediatophore), would still have to meet to form the fusion pore. The V0 domain of V-ATPase interacts in synaptic vesicles with the v-SNARE VAMP (Galli *et al.* 1996), and with the t-SNARE syntaxin at the plasma membrane (Shiff *et al.* 1996). It is therefore possible, as suggested by Peters *et al.* (2001) in yeast experiments, that, at the active zone also, the *trans*-SNARE complex acts by facilitating the pairing of the two opposite proteolipid rings; rather than forming the fusion pore itself (Weber *et al.* 1998). This would explain the inhibitory effect of SNARE knock-outs on the synchronized evoked transmitter release, while spontaneous release is only reduced (Broadie *et al.* 1995). Similarly, clostridial neurotoxins by cleaving the SNARE proteins block synaptic transmission while desynchronized quantal release persists (Dunant *et al.* 1987; Molgo *et al.* 1990).

In contrast to the collapse of synaptic vesicles in the plasma membrane, the kiss-and-run concept states that synaptic vesicles briefly interact with the plasma membrane and are retrieved at the same site (Fesce *et al.* 1994). In this process, transmitter release would occur without full fusion (Neher 1993) through a transient fusion pore (Almers and Tse 1990). Possibly, according to cytosolic Ca^{2+} concentration, the V0 *trans*-complex fusion pore may either flicker and close, or expand to full fusion (Alès *et al.* 1999). In

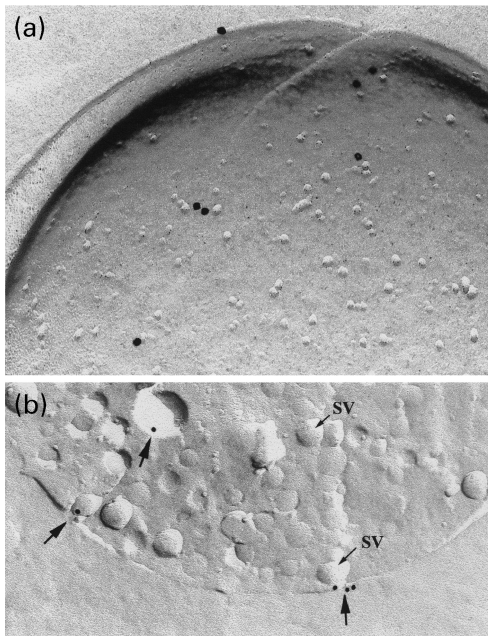

Fig. 3 Freeze fracture followed by immunolabelling of Torpedo electric organ synaptosomes (Taubenblatt *et al.* 1999). The V0 proteolipid subunit c was localized using monoclonal antibody 15K1. Surface view of the synaptosomal membrane (a) shows the frequent association of the 15 nm gold particles with large intramembrane particles. (b) The 15 nm gold particles (arrowheads) demonstrate the presence of the proteolipid at the docking site of synaptic vesicles (SV). Experiments performed in collaboration with T. Gulik-Krzywicki and J.C. Dedieu.

different cells or under different stimulation conditions, secretion could therefore use two different systems, a flickering pore, or exocytosis with full fusion. Figure 4 summarizes three modes of ACh release. After proteolipid reconstitution in liposomes (Israël *et al.* 1986; Peters *et al.* 2001), or expression in transfected cells (Falk-Vairant *et al.* 1996) or *Xenopus* oocytes (Cavalli *et al.* 1993), V0 proteolipids form hemichannels allowing, after activation by Ca^{2+}, the release of cytosolic ACh down its concentration gradient (Fig. 4, image 1). ACh could also be released through a fusion pore formed by the *trans*-pairing of two V0 domains of the V-ATPase (Fig. 4, image 2). *Trans*-SNARE complex would be important in this case to ensure a tight synaptic vesicle docking close to the active zone Ca^{2+} channels, and to center the V0 domains. The pore opening could be transient and ACh be released without full membrane fusion. In the third mode, the fusion pore would expand (Fig. 4, image 3), leading to membrane fusion and ACh release, with all vesicle contents. Subsequently, synaptic vesicles will have to be reformed by endocytosis. The latter mechanism is relatively slow and therefore unlikely to be at work in the most rapidly operating synapses.

Neurones may then use the same tool, the V0 sector of the V-ATPase, for proton transport, vesicle fusion and neurotransmitter release.

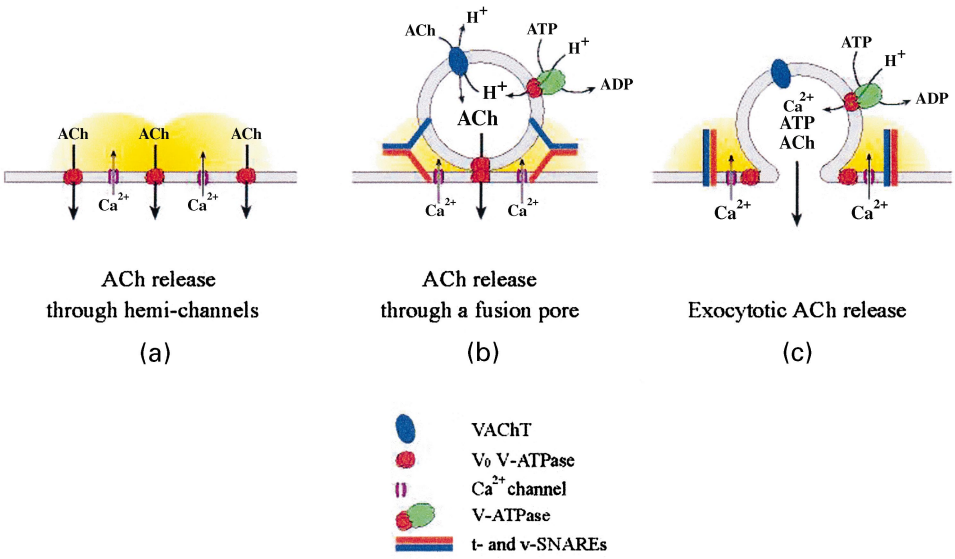

Fig. 4 Three modes of acetylcholine (ACh) release. (a) V0 proteolipids form hemichannels allowing, after activation by Ca^{2+}, the release of cytosolic ACh down its concentration gradient. This was shown to occur after proteolipid reconstitution in liposomes (Israël *et al.* 1986; Peters *et al.* 2001), or expression in *Xenopus* oocytes (Cavalli *et al.* 1993) or transfected cells (Falk-Vairant *et al.* 1996). (b) ACh release through a fusion pore formed by the *trans*-pairing of two V0 domains of the V-ATPase. *Trans*-SNARE complex would be important to ensure a tight synaptic vesicle docking close to the active zone Ca^{2+} channels, and to centre the V0 domains. The pore opening could be transient and ACh be released without full membrane fusion. (c) ACh release, with all vesicle contents, after expansion of the fusion pore and membrane fusion.

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