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Review

The fusion pore

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Abstract

The secretory process requires many different steps and stages. Vesicles must be formed and transported to the target membrane. They must be tethered or docked at the appropriate sites and must be prepared for fusion (priming). As the last step, a fusion pore is formed and the contents are released. Release of neurotransmitter is an extremely rapid event leading to rise times of the postsynaptic response of less than 100 μs. The release thus occurs during the initial formation of the exocytotic fusion pore. To understand the process of synaptic transmission, it is thus of outstanding importance to understand the molecular structure of the fusion pore, what are the properties of the initial fusion pore, how these properties affect the release process and what other factors may be limiting the kinetics of release. Here we review the techniques currently employed in fusion pore studies and discuss recent data and opinions on exocytotic fusion pore properties.

Keywords: Membrane fusion; Fusion pore; Exocytosis; Kiss-and-run; Transmitter release

1. Key landmarks in the history of fusion pores

Fusion pores play a crucial role in many cellular functions beyond exocytosis. All membrane translocations occurring in eukaryotic cells involve the formation of fusion pores. Through fusion pores, cells fuse lipid bilayers and target proteins from one cellular compartment to the next in membrane trafficking. In addition, fusion pores represent a key target for intracellular messenger regulation. Despite the continuous formation and dispersion of fusion pores inside cells, our knowledge about their structure and dynamic properties comes from studies of fusion pores formed at the plasma membrane during exocytosis. Morphological and imaging techniques can visualize fusion pores from the extracellular space (Fig. 1) and the patch clamp technique allows an electrophysiological characterization of their properties.

Chandler and Heuser [1] in 1980 reported the first image of an expanding fusion pore. Taking advantage of rapid-freezing techniques, they were able to capture fusion pores in degranulating mast cells. In a remarkable sequence of images from different pores, Chandler and Heuser showed membrane lined pores of 20–100 nm in diameter that provided a water path for secretory products to exit the vesicle interior to the extracellular space. Because of the large diameter and smoothness of the membrane lining the pores, at this point fusion pores seem to be made exclusively of lipids [1]. Similar images of fusion pores have been observed in other secretory systems, like Limulus amebocytes [2], neutrophils [3] and chromaffin cells [4]. More recently, the use of atomic force microscope provided new insights of the structure of fusion pores in living cells, corroborating the observation about fusion pores in fixed tissue with electron microscopy techniques [5]. Electron microscopy provides snapshots of fusion events. However, information about how fusion pores form and initially expand while their size is <20 nm escapes morphological analysis. Electrophysiological, electrochemical and fluorescence techniques provide information on fusion pores in the nanometer and sub-millisecond time scale.

The properties of early fusion pores have been investigated using biophysical techniques. Analogous to the characterization of single ion channels [6], the patch clamp technique revealed the properties of "early" fusion pores. Using admittance analysis to monitor changes in cell surface area to monitor exocytosis [7], the groups of Almers [8,9]

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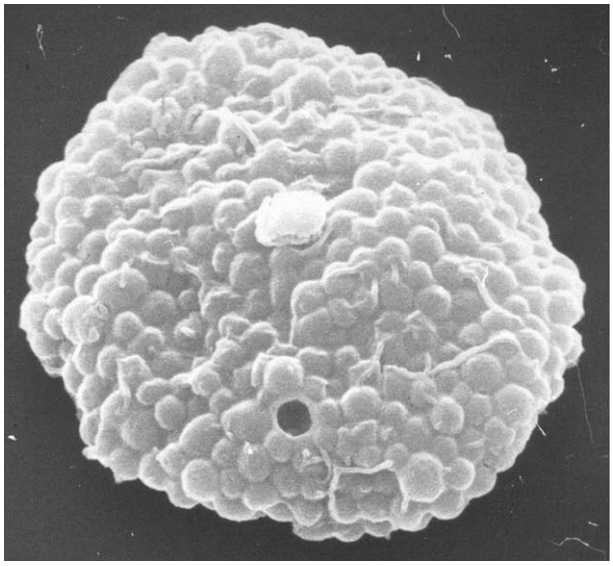

Fig. 1. Scanning electron micrograph of a rat peritoneal mast cells showing a widely open fusion pore through which the granular contents were released.

and Zimmerberg [10] independently derived the equivalent electrical circuit of degranulating beige mouse mast cells. These experiments provided the first estimations of fusion pore conductance and fusion pore kinetics in its early phases. In a remarkable study, Almers and coworkers concluded that the initial fusion pore conductance during exocytosis of beige mouse mast cells was about 200–300 pS, a conductance determined by a fusion pore that spans two lipid bilayers with a diameter of about 2 nm [9]. This value, similar to the conductance of a maxi K potassium channel or a gap junction channel, is consistent with a fusion pore made of proteins. Later experiments showed similar fusion pore conductances in eosinophils [11] and neutrophils [12] where initial fusion pore conductances with lower values, being as low as 50 pS, were detected by using the cell attached configuration. Because of the variability in initial fusion pore conductances, their erratic fluctuations, and the occurrence of net lipid transfer through transient fusion pores, Monck and Fernandez [13] proposed that fusion pores are lipidic. In this model, a scaffold of proteins attached to the vesicle and plasma membranes controls the properties of fusion pores. These two extreme hypotheses led to a debate still unresolved. There is consensus, however, that after a very short time, when the pore conductance exceeds 0.5–1 nS, the pore consists of a mixture of lipids and proteins [14]. Although the patch clamp technique and fluorescence methods have not yet provided direct evidence of the molecular nature of the fusion pore, these methods have provided much insight into the dynamics of pore formation, expansion and notably pore closure and the regulation by intracellular messengers.

In this review we discuss the techniques that are currently employed in fusion pore studies as well as recent data and opinions on exocytotic fusion pore properties. Considerable work has also been done on viral fusion, which will not be reviewed in detail here.

2. Electrophysiological methods for fusion pore measurements

Ion channel openings can be measured by dc current measurements across the membrane. However, dc current measurements through the fusion pore would require that one electrode is located inside the fusing vesicle, which so far has not been reported. However, currents charging the vesicle capacitance through the fusion pore can provide accurate measurements of fusion pore conductances. Fundamental to these measurements is that the membrane of the fusing vesicle may be represented by a capacitance C_V, connected to the extracellular space via the fusion pore with conductance G_P (Fig. 2A). Fusion of a single vesicle can be measured as a stepwise increase in membrane capacitance [7,15].

2.1. Current transients

In a whole cell patch clamp experiment, a known holding potential V_H is applied to the cell membrane. Before fusion an unknown potential V_0 may be present across the vesicle membrane. The vesicle potential relative to the bath is thus $V_V = V_H + V_0$. When a fusion pore opens, the vesicle capacitance C_V is discharged, leading to a brief current transient through the fusion pore [8]. By convention, the polarity is positive for outward currents and negative for inward currents. The integrated current provides the total charge Q_V required to discharge the vesicle capacitance providing

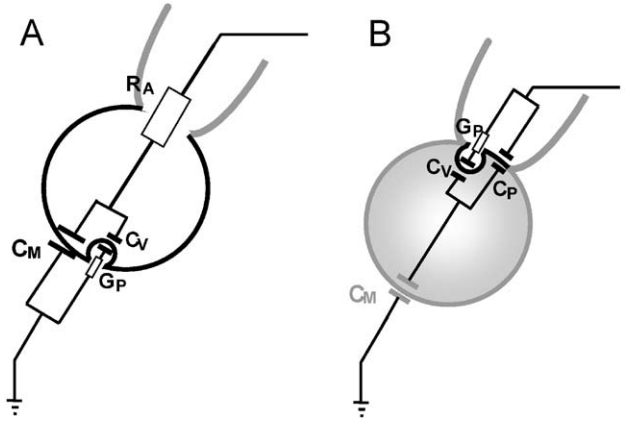

Fig. 2. Equivalent circuits for fusion pore analysis using the patch clamp technique in the whole-cell (A) and cell-attached (B) configurations. In the whole-cell configuration the pipette makes contact with the cell membrane (capacitance C_M) via the access resistance R_A. When a fusion pore is formed, the vesicle capacitance C_V is connected to the outside via fusion pore conductance G_P. In the cell-attached configuration C_M makes a negligible contribution and only the patch capacitance C_P and fusion events in the patch are measured.

$V_V = Q_V/C_V$. The intravesicular potential relative to the cytosol before fusion can then be calculated as $V_0 = Q_V/C_V - V_H$. Once the fusion pore opens, a current $I_P(t)$ begins to flow through the fusion pore. The intravesicular potential thus changes according to

$$V_V(t) = V_0 + V_H - \frac{1}{C_V}\int_0^t I_P(t')\mathrm{d}t'$$

and the time course of fusion pore conductance can be calculated as

$$G_P(t) = I_P(t)/V_V(t).$$

If G_P is constant while the discharge occurs, the transient has a single exponential time course with a time constant $\tau \sim C_V/G_P$. The applicability of this method requires sufficiently large granules such that sufficient charge is stored in the granule. The method has thus been used for beige mouse mast cells [8,16] and horse eosinophils [11], which have granules >1 μm in diameter. Typical values are $C_V = 100$ fF and $G_P = 200$ pS giving a charging time constant of 0.5 ms. These measurements thus reveal the properties of very early fusion pores during the first millisecond of their formation.

2.2. Admittance analysis

Following the discharge, continued measurements of fusion pore currents require changes in membrane potential such that C_V is charged and discharged repetitively, providing fusion pore currents that can be used to analyze the fusion pore conductance. Most measurements of this type have been performed applying a sine wave voltage to the cell membrane and analysis of the measured current with a lock-in amplifier in the piecewise linear mode [7,17,18]. When the fusion pore conductance changes from the unfused state ($G_P = 0$) to some value G_P, the admittance of the equivalent circuit (Fig. 2A) changes by

$$\Delta Y = T^2(\omega) \times \left[\frac{(\omega C_V)^2/G_P}{1 + (\omega C_V/G_P)^2} + i\frac{\omega C_V}{1 + (\omega C_V/G_P)^2}\right]$$

[19]. Besides of a scaling factor and phase shift in the factor $T^2(\omega)$, the admittance change has a real part

$$\mathrm{Re} = \frac{(\omega C_V)^2/G_P}{1 + (\omega C_V/G_P)^2} \quad \text{and an imaginary part}$$

$$\mathrm{Im} = \frac{\omega C_V}{1 + (\omega C_V/G_P)^2}$$

When the pore has expanded to a large conductance, Im approaches ωC_V adding the vesicle capacitance to the cell capacitance [7,17]. Knowing C_V, G_P can be calculated from Re or Im [8,10,16]. Alternatively, Re and Im can be used to calculate directly C_V and G_P as

$$\omega C_V = \frac{\mathrm{Re}^2 + \mathrm{Im}^2}{\mathrm{Im}} \quad \text{and} \quad G_P = \frac{\mathrm{Re}^2 + \mathrm{Im}^2}{\mathrm{Re}}$$

Depending on the desired time resolution, this method can be employed to measure fusion pore conductances in smaller vesicles. The time resolution of these measurements is typically about 10 ms. In whole cell experiments fusion pore conductances in 500-nm diameter vesicles could be well resolved in rat mast cells and guinea-pig eosinophils [20,21]. For smaller vesicles like those present in neutrophils or chromaffin cells, whole cell measurements are usually too noisy to resolve single fusion events as clear capacitance steps. The sources of this noise are inherent to the recording configuration and are discussed in detail elsewhere [17,18,22].

2.3. Cell-attached measurements

Low noise single channel recordings are performed using cell-attached or excised patches [6] and corresponding low noise capacitance measurements can be performed in the cell-attached configuration (Fig. 2B) [7]. In patch recordings, fusion pore measurements can be performed by admittance analysis analogous to the whole cell methods described above [12,23,24]. A detailed description of the method has been published [24]. The main difference is that to resolve fusion pore conductances in the relevant range, higher sine wave frequencies are applied (typically about 20 kHz). The method can also be applied to study fusion pores in cell-free excised patches [25]. With this method, fusion pore analysis was performed for neutrophil granules [12,23], chromaffin granules [26,27] and even synaptic-like microvesicles in PC12 cells [28].

2.4. Amperometric recordings

The time course of transmitter release from single vesicles can be measured by amperometry using carbon fiber electrodes [29–31]. A distinct feature of many events is the so-called ‘foot signal’ that often precedes the amperometric spike. It was originally demonstrated in mast cells that foot signals reflect slow release of vesicular contents through a narrow fusion pore [32]. The duration of a foot signal indicates the time interval during which the fusion pore is small, allowing release only at a low rate. It can thus be used as an indicator for the time interval between pore formation and expansion. In principle, the amperometric current during the foot indicates the flux rate of molecules through the pore and could thus also contain information about fusion pore size. However, since amperometry alone does not allow determination of free transmitter concentration in single vesicles, the ‘calibration’ to convert amperometric foot currents into fusion pore con-

ductances is not straightforward. Recently, amperometric recordings have been performed using a four-electrode array of platinum electrodes patterned on a glass coverslip [33]. This 'electrochemical imaging' method provides not only temporal but also spatial information indicating time and position of individual fusion pore openings.

In several studies the time course of amperometric spikes (as opposed to foot signals) has been investigated. Effects on quantal size, the number of molecules released per event (spike charge) and on the width of amperometric spikes have been taken as evidence for changes in fusion pore properties [34–37]. However, amperometrically measured quantal size is also modulated by treatments that affect loading of vesicles with transmitter [38] and the time course of release as well as quantal size are also affected by dissociation of transmitter from the vesicular matrix [39]. The relation between features of amperometric spikes and fusion pore properties has not been demonstrated and is presently hypothetical.

2.5. Patch amperometry

Combined capacitance and amperometric measurements provide simultaneously an indication of fusion and fusion pore conductance, as well as the rate of transmitter release from the same vesicle. In whole cell recordings this can be used for cells with large vesicles such as mast cells [32]. To simultaneously measure fusion (by capacitance) and release (by amperometry) in chromaffin cells, cell-attached capacitance measurements have been combined with amperometric detection of catecholamine release inside the patch pipette [26]. This method, named 'patch amperometry', uses a carbon fiber electrode inside the patch pipette in addition to the conventional pipette electrode [26]. The method allows a detailed characterization of fusion pore properties and their role in determining the dynamics of release [26,27,40].

3. Fluorescence measurements

Fusion can be investigated by a wide variety of fluorescent probes, which will not be reviewed extensively here. Some fluorescence techniques have been employed to obtain information on fusion pore properties. One widely used probe is the lipophilic dye FM1-43 [41]. Although soluble in water, FM1-43 partitions preferentially into membranes where it is highly fluorescent. When vesicles are recycled, the dye is taken up in their membrane and can be released upon subsequent stimulation. FM1-43 and its analogs are widely used to study vesicle recycling and have also been used to investigate fusion pore properties. FM dyes dissociate slowly from the membrane such that only a fraction of it is released when a fusion pore opens for only a brief period [42]. Different FM dyes with different dissociation rate constants have been used [43]. While destaining of a vesicle is slow, transmitter release from the same vesicle is complete within a fraction of a millisecond and should not change as long as fusion pore open times exceed this time scale.

4. Fusion pore structure and function

Properties of individual single exocytotic fusion events were originally studied on mast cells from *beige mice*, which have unusually large granules allowing an electrophysiological characterization [8–10,16]. These experiments revealed the opening of a narrow fusion pore with a conductance similar to that of large ion channels or gap junctions [8,16]. Normal horse eosinophils have similarly large granules. In these cells the initial fusion pore conductances are similar to those in beige mouse mast cells. The initial fusion pore conductance does not depend on the intracellular calcium concentration [11]. The rate of fusion pore expansion was studied in detail in horse eosinophils using whole cell capacitance measurements. It was observed that the expansion rate was regulated by the intracellular calcium concentration [11,44] as well as the phorbol ester PMA [45], implicating phosphorylation by protein kinase C or another target of PMA.

Little is known about the identity of SNARE proteins mediating exocytosis in mast cells and granulocytes. In neurons and chromaffin cells, however, the SNARE proteins VAMP, syntaxin and SNAP-25 are thought to form and expand the fusion pore. The dependence of membrane fusion on the concentration of soluble VAMP2 coil domains has suggested that three SNARE complexes mediate fusion of a vesicle [46]. In PC12 cells, overexpression of synaptotagmin I prolonged the lifetime of the narrow fusion pore measured as amperometric foot signal duration, whereas synaptotagmin IV shortened this time. Both synaptotagmin isoforms reduced norepinephrine flux through open fusion pores [47].

5. Full fusion or kiss-and-run

The question if transmitter release from synaptic vesicles is associated with full incorporation of the vesicle into the plasma membrane or if only a transient fusion pore is formed under physiological conditions has been controversial for many years [48–50]. Full fusion means that following its formation the fusion pore expands to a large size and the vesicle membrane becomes fully incorporated into the plasma membrane. Transient fusion means that the pore opens and maybe expands but then closes again such that the vesicle retains its integrity when it discharges its contents (kiss-and-run). More recent evidence for kiss-and-run fusion of synaptic vesicles has come from experiments with FM dyes using the kinase inhibitor staurosporine [42]. However, the data concerning kiss-and-run are conflicting

as it seems that kiss-and-run may be occurring under certain conditions and the significance of kiss-and-run may also vary among cell types.

Full fusion and kiss-and-run fusion can be directly distinguished using capacitance measurements. Full vesicle incorporation into the plasma membrane produces a capacitance step, whereas kiss-and-run fusion produces a capacitance flicker [15,32]. Using patch amperometry [26] it has become possible to directly observe kiss-and-run fusion in chromaffin cells. It was found that the nature of exocytotic events is shifted from permanent fusion to kiss-and-run when the (extracellular) calcium concentration in the pipette is strongly elevated. At 80 mM Ca^{2+} in the cell-attached pipette, most events were fast kiss-and-run events with a mean duration of only about 60 ms [27]. A preference for kiss-and-run was recently also observed in chromaffin cells following staurosporine treatment [51]. In hippocampal synapses using the membrane staining FM-dyes, it was concluded that internalized vesicles are not, as the classical picture suggests, recycled via endosome-like compartments, but are instead recycled directly [52]. Measurements of the destaining kinetics suggested that synaptic vesicles fuse only transiently and that the duration of the fused state may strongly depend on calcium [43]. An extremely rapid mode of kiss-and-run (<6 ms) was observed, which was independent of presynaptic calcium concentrations but was supported by hypertonic solution [53]. In synaptic terminals of bipolar neurons, fast endocytosis is also activated by calcium influx [54]. Using a pH-sensitive GFP–VAMP construct, it was found that the time constant of endocytosis increases with the extent of exocytosis stimulated. It was concluded that only one mechanism exists for endocytosis, which is saturable, retrieving about 1 vesicle/s [55]. Experiments on the calyx of held also showed that the rate of endocytosis decreases with the number of fused vesicles and was shown to be independent on the presynaptic calcium concentration [56]. Using simultaneous measurement of postsynaptic currents and nerve terminal capacitance, it was found that under weak stimulation conditions, kiss-and-run is the dominant mode with a lifetime of the fused state of about 60 ms. On the other hand, studies using FM 1-43 in synapses of bipolar neurons suggested that kiss-and-run is not a significant mechanism of transmitter release in these cells [57].

In some studies carbon fiber amperometry was used and changes in quantal size and spike duration were taken as evidence for kiss-and-run with incomplete release. Such effects were observed following PMA treatment [34] or interference with dynamin [35] and following overexpression of the R39C mutant of munc18 [36] or the protein complexin [37]. It has been argued that kiss-and-run might be a matter of competence, which means that the ability to undergo rapid endocytosis may be a property preacquired by a vesicle similar to the way priming makes a vesicle readily releasable [58]. Alternatively, phosphorylation has been proposed as a switch between kiss-and-run and full fusion [59]. However, it is unclear at present why staurosporine as well as PMA treatment both shift the mode of exocytosis to kiss-and-run.

Rapid endocytosis is often considered equivalent to kiss-and run although direct evidence is usually not available. Rapid endocytosis may involve dynamin [60] but may also be enabled via a dynamin-independent mechanism [35]. Our own experiments have shown that release during fast kiss-and-run of chromaffin granules is complete [27], which is not consistent with a change in spike duration and quantal size due to kiss-and-run fusion. Cell-attached capacitance measurements have also revealed capacitance decrease following exocytosis of a single vesicle that did not reflect reclosing of the fusion pore [61]. Thus it cannot be excluded that a reversible capacitance change may reflect a tight coupling of exocytosis and endocytosis but not necessarily reversal of fusion pore opening. Fluorescence imaging of PC12 membrane sheets revealed that $\sim 30\%$ of exocytosed vesicles are recaptured. The recapturing was not simple fusion pore flickering but involved dynamin, indicating a tight coupling of exo- and endocytosis [62].

6. Fusion pore models and future perspectives

Although at present no clear molecular model exists for the fusion pore, it is widely believed that pore formation is a consequence of structural changes in the SNARE complex. Present models of the fusion pore envision the C-terminal ends of VAMP and syntaxin to be located close to each other right in the center of the pore [63,64]. The C-terminal end of SNAP-25 is located near the putative location of the transmembrane domains of VAMP and syntaxin. Deletions of four to nine amino acids at the C terminus of SNAP-25 reduce exocytosis in chromaffin cells [65,66] and several point mutations in this domain show some inhibition and effects on amperometric spike shapes [67]. The SNARE hypothesis of fusion suggests that the initial fusion pore may be a proteolipid structure. There is considerable evidence for exocytosis as well as viral fusion that enlarged pores allow bulk lipid flow, whereas small pores do not [61,68,69]. If the pore is indeed formed by a small number of SNARE complexes, it appears unlikely that the core of the pore involves bulk lipid.

In addition to the SNARE complex, the free V0 part of the proton ATPase has been implicated in fusion pore formation of yeast vacuoles [70], which is also reminiscent of older findings on what was at that time called the mediatophore [71]. V0 trans-complexes may form a proteolipid-lined channel at the fusion site and radial expansion of such a protein pore may be a mechanism for membrane fusion [70]. The proton ATPase should thus also be considered as a possible component of the fusion pore. It has been demonstrated that lipid vesicles containing the appropriate pairs of v-SNAREs and t-SNAREs fuse, indicating that SNAREs alone are sufficient to form fusion pores [63].

However, fusion can even be demonstrated in pure lipid vesicles free of protein. It will thus be necessary to demonstrate that these reconstituted fusion pores have the right properties. The challenge in elucidating the molecular mechanism of fusion resembles the challenges in elucidating the opening of ion channels. Future work will need to combine electrophysiological fusion pore measurements with molecular biology as well as fusion pore measurements in reconstituted systems. Such studies may hopefully provide the information needed to achieve a mechanistic molecular understanding of fusion pore formation and expansion.

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References

[1] D. Chandler, J. Heuser, J. Cell Biol. 86 (1980) 666–674.
[2] R.L. Ornberg, T.S. Reese, J. Cell Biol. 90 (1981) 40–54.
[3] T. Nakata, K. Sobue, N. Hirokawa, J. Cell Biol. 110 (1990) 13–25.
[4] W. Schmidt, A. Patzak, G. Lingg, H. Winkler, Eur. J. Cell Biol. 32 (1983) 31–37.
[5] S.W. Schneider, K.C. Sritharan, J.P. Geibel, H. Oberleithner, B.P. Jena, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 316–321.
[6] O.P. Hamill, A. Marty, E. Neher, B. Sakmann, F.J. Sigworth, Pflügers Arch. Eur. J. Physiol. 391 (1981) 85–100.
[7] E. Neher, A. Marty, Proc. Natl. Acad. Sci. U. S. A. 79 (1982) 6712–6716.
[8] L.J. Breckenridge, W. Almers, Nature 328 (1987) 814–817.
[9] L.J. Breckenridge, W. Almers, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 1945–1949.
[10] J. Zimmerberg, M. Curran, F.S. Cohen, M. Brodwick, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 1585–1589.
[11] J. Hartmann, M. Lindau, FEBS Lett. 363 (1995) 217–220.
[12] K. Lollike, N. Borregaard, M. Lindau, J. Cell Biol. 129 (1995) 99–104.
[13] J.R. Monck, J.M. Fernandez, J. Cell Biol. 119 (1992) 1395–1404.
[14] M. Lindau, W. Almers, Curr. Opin. Cell Biol. 7 (1995) 509–517.
[15] J.M. Fernandez, E. Neher, B.D. Gomperts, Nature 312 (1984) 453–455.
[16] A.E. Spruce, L.J. Breckenridge, A.K. Lee, W. Almers, Neuron 4 (1990) 643–654.
[17] M. Lindau, E. Neher, Pflügers Arch. Eur. J. Physiol. 411 (1988) 137–146.
[18] K.D. Gillis, in: B. Sakmann, E. Neher (Eds.), Single-Channel Recording, 2nd ed., Plenum, New York, 1995, pp. 155–198.
[19] M. Lindau, Q. Rev. Biophys. 24 (1991) 75–101.
[20] M.J. Curran, F.S. Cohen, D.E. Chandlers, P.J. Munson, J. Zimmerberg, J. Membr. Biol. 133 (1993) 61–75.
[21] M. Lindau, O. Nüße, J. Bennett, O. Cromwell, J. Cell. Sci. 104 (1993) 203–209.
[22] A. Marty, E. Neher, in: B. Sakmann, E. Neher (Eds.), Single Channel Recording, 2nd ed., Plenum, New York, 1995, pp. 31–52.
[23] K. Lollike, M. Lindau, J. Immunol. Methods 232 (1999) 111–120.
[24] K. Debus, M. Lindau, Biophys. J. 78 (2000) 2983–2997.
[25] G. Dernick, G. Alvarez de Toledo, M. Lindau, Biophys. J. 76 (1999) A68.
[26] A. Albillos, G. Dernick, H. Horstmann, W. Almers, G. Alvarez de Toledo, M. Lindau, Nature 389 (1997) 509–512.
[27] E. Alés, L. Tabares, J.M. Poyato, V. Valero, M. Lindau, G. Alvarez de Toledo, Nat. Cell Biol. 1 (1999) 40–44.
[28] V.A. Klyachko, M.B. Jackson, Nature 418 (2002) 89–92.
[29] R.M. Wightman, J.A. Jankowski, R.T. Kennedy, D.T. Kawagoe, T.J. Schroeder, D.J. Leszczyszyn, J.A. Near, E.J. Diliberto Jr., O.H. Viveros, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 10754–10758.
[30] R.H. Chow, L.V. Rüden, E. Neher, Nature 356 (1992) 60–63.
[31] R.H. Chow, L.V. Rüden, in: B. Sakmann, E. Neher (Eds.), Single Channel Recording, Plenum, New York, 1995, pp. 245–275.
[32] G. Alvarez de Toledo, R. Fernández-Chacón, J.M. Fernandez, Nature 363 (1993) 554–558.
[33] A.F. Dias, G. Dernick, V. Valero, M.G. Yong, C.D. James, H.G. Craighead, M. Lindau, Nanotechnology 13 (2002) 285–289.
[34] M.E. Graham, R.J. Fisher, R.D. Burgoyne, Biochimie 82 (2000) 469–479.
[35] M.E. Graham, D.W. O'Callaghan, H.T. McMahon, R.D. Burgoyne, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 7124–7129.
[36] R.J. Fisher, J. Pevsner, R.D. Burgoyne, Science 291 (2001) 875–878.
[37] D.A. Archer, M.E. Graham, R.D. Burgoyne, J. Biol. Chem. 277 (2002) 18249–18252.
[38] D. Sulzer, E.N. Pothos, Rev. Neurosci. 11 (2000) 159–212.
[39] R.M. Wightman, K.P. Troyer, M.L. Mundorf, R. Catahan, Ann. N. Y. Acad. Sci. 971 (2002) 620–626.
[40] L. Tabares, E. Ales, M. Lindau, G. Alvarez De Toledo, J. Biol. Chem. 276 (2001) 39974–39979.
[41] W.J. Betz, F. Mao, G.S. Bewick, J. Neurosci. 12 (1992) 363–375.
[42] A.W. Henkel, W.J. Betz, J. Neurosci. 15 (1995) 8246–8258.
[43] J. Klingauf, E.T. Kavalali, R.W. Tsien, Nature 394 (1998) 581–585.
[44] R. Fernández-Chacón, G. Alvarez de Toledo, FEBS Lett. 363 (1995) 221–225.
[45] S. Scepek, J.R. Coorssen, M. Lindau, EMBO J. 17 (1998) 4340–4345.
[46] Y. Hua, R.H. Scheller, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 8065–8070.
[47] C.T. Wang, R. Grishanin, C.A. Earles, P.Y. Chang, T.F. Martin, E.R. Chapman, M.B. Jackson, Science 294 (2001) 1111–1115.
[48] J.E. Heuser, T.S. Reese, J. Cell Biol. 57 (1973) 315–344.
[49] B. Ceccarelli, W.P. Hurlbut, A. Mauro, J. Cell Biol. 57 (1973) 499–524.
[50] B. Ceccarelli, W.P. Hurlbut, A. Mauro, J. Cell Biol. 54 (1972) 30–38.
[51] A.W. Henkel, G. Kang, J. Kornhuber, J. Cell. Sci. 114 (2001) 4613–4620.
[52] C.N. Murthy, C.F. Stephens, Nature 392 (1998) 497–501.
[53] C.F. Stevens, J.H. Williams, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 12828–12833.
[54] G. Neves, A. Gomis, L. Lagnado, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 15282–15287.
[55] S. Sankaranarayanan, T.A. Ryan, Nat. Cell Biol. 2 (2000) 197–204.
[56] J.Y. Sun, X.S. Wu, L.G. Wu, Nature 417 (2002) 555–559.
[57] D. Zenisek, J.A. Steyer, M.E. Feldman, W. Almers, Neuron 35 (2002) 1085–1097.
[58] F. Valtorta, J. Meldolesi, R. Fesce, Trends Cell Biol. 11 (2001) 324–328.
[59] R.D. Burgoyne, R.J. Fisher, M.E. Graham, Trends Cell Biol. 11 (2001) 404–405.
[60] C.R. Artalejo, A. Elhamdani, H.C. Palfrey, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 6358–6363.
[61] K. Lollike, N. Borregaard, M. Lindau, Biophys. J. 75 (1998) 53–59.
[62] P. Holroyd, T. Lang, D. Wenzel, P. De Camilli, R. Jahn, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16806–16811.
[63] T. Weber, B.V. Zemelman, J.A. McNew, B. Westermann, M. Gmachl, F. Parlati, T.H. Söllner, J.E. Rothman, Cell 92 (1998) 759–772.
[64] R.B. Sutton, D. Fasshauer, R. Jahn, A.T. Brunger, Nature 395 (1998) 347–353.
[65] S. Wei, T. Xu, U. Ashery, A. Kollewe, U. Matti, W. Antonin, J. Rettig, E. Neher, EMBO J. 19 (2000) 1279–1289.

[66] M. Criado, A. Gil, S. Viniegra, L.M. Gutierrez, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 7256–7261.
[67] A. Gil, L.M. Gutierrez, C. Carrasco-Serrano, M.T. Alonso, S. Viniegra, M. Criado, J. Biol. Chem. 277 (2002) 9904–9910.
[68] J.R. Monck, G. Alvarez de Toledo, J.M. Fernandez, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 7804–7808.
[69] F.W. Tse, A. Iwata, W. Almers, J. Cell Biol. 121 (1993) 543–552.
[70] C. Peters, M.J. Bayer, S. Buhler, J.S. Andersen, M. Mann, A. Mayer, Nature 409 (2001) 581–588.
[71] M. Israel, N. Morel, B. Lesbats, S. Birman, R. Manaranche, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 9226–9230.