

Peeping at the vesicle kiss

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Kiss-and-run fusion, a quick way of coupling exocytotic vesicle fusion to vesicle recycling by endocytosis, can allow full discharge of secretory-vesicle contents, such as neurotransmitters, and is more common than classical endocytosis at high calcium concentrations.

Release of secretory-vesicle cargo into the extracellular space by the process of exocytosis involves the fusion of these secretory vesicles with the plasma membrane. As cells need to stay the same size, the membrane that was added to the plasmalemma by the vesicles must be internalized by endocytosis. How is this membrane recycling achieved? Several conflicting theories have been proposed, from the classical endocytotic cycle, which involves full-blown fusion of the secretory vesicle with the membrane and then the pinching off and internalization of a new vesicle¹, to quick local recycling following reversible fusion² or 'kiss-and-run'³. Reversible membrane fusion has been reported to occur through the transient opening of a small aqueous pore^{4–6}, but could such a rapid process be important in secretion? Alés and colleagues, in a paper on page 40–44 of this issue⁷, answer this question convincingly for chromaffin cells: full discharge of the content of secretory vesicles can occur through kiss-and-run reversible fusion, and calcium favours the process. This is the first clear-cut demonstration that multiple pathways are available for the ves-

icle to discharge its content and recycle, with the relative importance of each path being regulated by calcium and possibly other factors (Fig. 1).

The conventional view of vesicle recycling, proposed in the 1970s by Heuser and Reese¹, holds that, at nerve terminals, exocytosis and endocytosis are two separate processes: vesicle endocytosis is initiated at the flat plasma membrane — after the full fusion and merging of the secretory-vesicle membrane with the plasma membrane — by the sorting of specific vesicle components, assisted by a protein 'cage'. The resulting protein-coated vesicles would carry the membrane to local endosomes, from which new synaptic vesicles would be generated.

Although the rates of such a recycling pathway initially appeared to be too slow for neurons, later studies have shown that the process is much faster at synapses (time constants between 20 and 30 seconds) than in other secretory cells. Moreover, synaptic vesicles can regenerate directly from the plasmalemma, the trip to the endosome being unnecessary^{8,9}. General opinion converged on the two-step interpretation of vesicle recycling, which is still reported in

most textbooks as the only possible way in which vesicle internalization can take place at synapses.

But many years ago Ceccarelli and colleagues² proposed that fused vesicles might simply detach from the plasma membrane after exocytosis, quickly enough to prevent their collapse and the dispersal of their specific components. Morphological and immunocytochemical evidence in support of this theory has accumulated³. Moreover, measurements of membrane capacitance in mast and chromaffin cells showed that a small aqueous pore (with a conductance measuring less than 1 nanosiemens) forms when a vesicle fuses with the membrane^{4–6}. The pore opening often flickers for a short time before either closing or irreversibly dilating to produce vesicle collapse. In micro-amperometric assays of released amines, these events are paralleled by a small deflection (foot) in the current, indicating some 'trickling' of the amines out of the pore; the current then either aborts or evolves into a full spike, indicating full discharge^{4–6}. Finally, heterogeneity of endocytosis has been reported in chromaffin cells, with fast components (of 5 seconds in

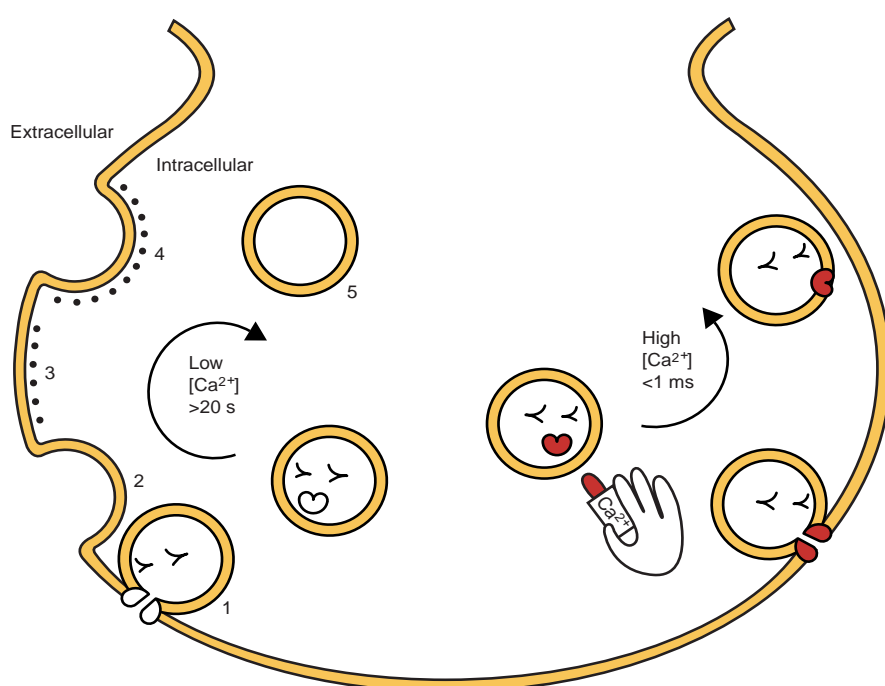


Figure 1 Two models of coupled exocytosis–endocytosis. The conventional process (left) predominates at low Ca²⁺ concentrations. After fusion of the vesicle with the plasma membrane, an aqueous pore is formed (1), followed by flattening of the vesicle in the plasma membrane (2). Sorting of specific components (3) and retrieval of the vesicle membrane (4) are accomplished through the formation of a clathrin coat. The recycled vesicle sheds its coat upon detaching from the membrane (5) and is then refilled to become available for a new run of discharge. The average time for vesicle fusion and retrieval is several tens of seconds. Kiss-and-run fusion (right) is favoured in high Ca²⁺ levels. The vesicle fuses briefly with the plasma membrane, and widening of the pore — sufficient for the full discharge of vesicle content — ensues. Quick resealing and recycling of the vesicle then follow. In synapses, this type of exocytotic–endocytic cycle might take less than 1 millisecond.

duration) occurring during strong cellular stimulation^{10,11}. However, the frequency of reversible kiss-and-run openings, and their relevance to secretion, remained uncertain.

This is where the work of Alés and colleagues⁷ comes in. They show, in contrast with previous results, that, in chromaffin cells, the release of the whole vesicle cargo does not require full-blown fusion. In fact, the reversible fusion pore can open up to a size above 1.5 nanosiemens, enough to discharge the full contents of the vesicle, and can still close quickly. This means that kiss-and-run exocytosis–endocytosis can sustain quantal neurotransmitter release.

How did Alés *et al.* obtain these new results? They used the powerful approach of coupling measurements of cell-attached membrane capacitance to assays of catecholamine, a neurotransmitter, by using a carbon fibre positioned within the patch-clamp pipette; but their success is also based on their use of different extracellular Ca²⁺ concentrations. As they increased the Ca²⁺ concentration from 5 to 90 mM, the frequency of reversible fusion events rose from 5% to almost 80%; still, in most cases, the full vesicle content was discharged. Thus, these cells appear to be able to choose between at least two routes for membrane recycling: conventional endocytosis predominates when Ca²⁺ levels are low, and kiss-and-run fusion is the preferred method in high Ca²⁺ concentrations. As well as inducing kiss-and-run, high Ca²⁺ amounts also decrease the duration of the process. These two effects of calcium might in fact be related to the same molecular mechanism — the increased probability of pore resealing.

Alés *et al.*⁷ applied Ca²⁺ extracellularly. But they assumed that its regulatory effect took place intracellularly, and the very high concentrations used were aimed at mimick-

ing the state of intensely stimulated neurons, in which massive amounts of exocytosis occur and quick recycling, as can be sustained by kiss-and-run, is needed. It remains possible, however, that regulation by Ca²⁺ also takes place at the external face of the membrane. This might be particularly relevant at synapses. During exocytosis, in fact, the synaptic cleft, which is often very thin, is flooded by not only the neurotransmitter but also the entire vesicle cargo, including Ca²⁺, which is present in very high amounts (tens of millimoles per litre) inside secretory vesicles.

Are these results, obtained in chromaffin cells, relevant to synaptic function? It is interesting that chromaffin granules, although considerably larger than clear synaptic vesicles, resemble the other type of synaptic secretory organelle, that sustaining transmission of peptide neurotransmitters. And the molecular apparatus for exocytosis, including SNARE proteins and the entire fusion machinery, is largely similar in the two cell types. Last but not least, the time barrier of 1 millisecond, which has so far somewhat demarcated synaptic from non-neuronal secretion, is closely approached by kiss-and-run fusion in chromaffin cells. If a chromaffin granule, which has a volume about ten times that of a synaptic secretory vesicle and is filled with an organized macromolecular matrix that traps the amine neurotransmitters, can be fully discharged in a few milliseconds, it is quite conceivable that a synaptic vesicle could release its soluble neurotransmitter in a fraction of a millisecond and then rapidly pinch off from the presynaptic membrane.

The most convincing argument in support of the coherence between chromaffin cells and neurons stems from the comparison of Alés *et al.*'s findings⁷ with those reported last year by Klingauf *et al.*¹², who

studied hippocampal neurons.

Klingauf *et al.*¹² used a completely different approach to that of Alés and colleagues: they preloaded synaptic terminals with styryl dyes, which become incorporated into membranes. By comparing three dyes that are characterized by distinct rates of depolarization, Klingauf and colleagues were able to reveal the existence of at least two components of recycling — a slow process, with a time constant in the order of 20–30 seconds, and a much faster process, which they interpreted to be kiss-and-run recycling. Most relevant, the occurrence of the latter process increased greatly with stimulus strength and higher Ca²⁺ amounts. Staurosporin, a nonspecific kinase blocker, speeded up the recycling, pointing to the involvement of further signalling mechanisms in the regulation of kiss-and-run.

With their approach, however, Klingauf *et al.*¹² could estimate the kinetics of pore closure only indirectly (they calculated the lifetime of the pore to be less than 2 seconds) and, more important, they could not prove that kiss-and-run events produce functionally relevant release of neurotransmitter. This proof is now provided by the results of Alés *et al.*⁷

Calcium has long been known to be the major factor in regulating exocytosis. Alés and colleagues⁷ have now shown that this ion has a central function in regulating the whole exocytotic–endocytotic cycle, by making kiss-and-run the preferred recycling path. Huge Ca²⁺ concentrations are needed to regulate this very rapid process, but Ca²⁺ concentration is known to rise to tremendous levels at the presynaptic membrane during stimulus-evoked neurotransmitter release. So, when the action potential invades the nerve terminal and Ca²⁺ rushes in, the neuron under pressure has an efficient way to communicate across the synapse: a quick kiss! □

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1. Heuser, J. E. & Reese, T. S. *J. Cell Biol.* 57, 315–344 (1973).
2. Ceccarelli, B., Hurlbut, W. P. & Mauro, A. *J. Cell Biol.* 57, 499–524 (1973).
3. Fesce, R., Grohovaz, F., Valtorta, F. & Meldolesi, J. *Trends Cell Biol.* 4, 1–4 (1994).
4. Neher, E. *Nature* 363, 497–498 (1993).
5. Alvarez de Toledo, G., Fernandez-Chacon, R. & Fernandez, J. M. *Nature* 363, 554–558 (1993).
6. Henkel, A. W. & Almers, W. *Curr. Opin. Neurobiol.* 6, 350–357 (1996).
7. Alés, E. *et al.* *Nature Cell Biol.* 1, 40–44 (1999).
8. Takei, K., Mundig, O., Daniell, L. & De Camilli, P. *J. Cell Biol.* 133, 1237–1250 (1996).
9. Murthy, V. N. & Stevens, C. F. *Nature* 392, 497–501 (1998).
10. Smith, C. & Neher, E. *J. Cell Biol.* 139, 885–894 (1997).
11. Engisch, K. L. & Nowycky, M. *J. Physiol. (Lond.)* 506, 591–608 (1998).
12. Klingauf, J., Kavalali, E. T. & Tsien, R. W. *Nature* 394, 581–585 (1998).