New and Notable

Release of Secretory Vesicle Contents: Regulation after Fusion?

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A ubiquitous problem in cell biology concerns the release of secretory vesicle contents into extracellular space. Such release is the fundamental mechanism for chemical communication between cells. In spite of immense efforts, as many mysteries surround the mechanism of release as do certainties. In the past few years, however, two relatively new techniques have made significant advances: the measurement of membrane capacitance jumps as vesicles fuse to the plasma membrane and amperometric measurements that directly monitor secretory products. The addition of secretory vesicle membranes to the cell membrane measurably increases the capacitance of the cell and thus visualizes in real time the process of fusion. The fast time resolution of the capacitance jump experiments has suggested that the delay between cell stimulation by secretagogues and vesicle fusion itself takes place in a few milliseconds. However, this interpretation relies on an equivalent circuit of the cell, which is assumed to be independent of the stimulation and the secretory events. Furthermore, the resolution of the capacitance data restricts the measurement of individual events to vesicles larger than 200 nm in diameter (Robinson et al., 1995). The second method applies to oxidizable secretions such as catecholamines and serotonin. A carbon fiber (held at 650 mV, sufficient to oxidize serotonin, for example) placed near a secreting cell can record serotonin as a current. Because amperometry directly measures the chemical messenger, the method is unaffected by factors that complicate capacitance measurements. Furthermore, amperometry can resolve vesicle fusion events that are below the limit of capacitance jump recordings. The area under the amperometric spike measures the number of released molecules, providing a useful comparison with the events measured by capacitance jumps (von Rüden and Neher, 1993). Recently, Oberhauser et al. (1996) have shown in isolated mast and chromaffin cells, well-studied models for vesicular release mechanisms, that the method of stimulation has profound effects on the kinetics of fusion as recorded by these two methods. In response to GTPγS the two methods are essentially equivalent. However, the release of caged Ca inside mast or chromaffin cells shows a prompt graded increase in capacitance with no initial concomitant increase in secretion as measured by amperometry.

This result suggests that caution be taken in interpreting the capacitance data in terms of chemical release. In particular, it suggests that vesicles may have mechanisms to restrict the release of their contents even though membrane fusion has occurred. In this issue of the *Biophysical Journal*, Pihel et al. (1996) have helped unravel the mechanism of chemical release by studying two important factors that are likely to contribute to secretion: the physical state of the matrix that contains the secretory products and the temperature at which secretion occurs. Using digitonin, a membrane permeabilizing agent that promotes secretion, Pihel et al. report that in the presence of Zn^{2+}, less serotonin is released from mast cells, whereas the opposite is true for Cs^{+}. The interpretation of these results is complex; however, it involves the notion that the interiors of granules have properties similar to an ion-exchange gel (Uvnäs and Aborg, 1983). A pure ion exchange mechanism involves simple electroneutrality, so it is not obvious why divalents and monovalents act differently. Pihel et al. suggest that Zn ions cross-link the gel more strongly than Cs ions, resulting in rates of dissociation that either diminish or promote exocytosis. Although details are wanting, the idea is clear: molecules like serotonin do not merely float around in the vesicle ready for release when fusion occurs. Rather they are stored in a gel that regulates their release, and the mechanism of storage looks increasingly electrostatic. The idea is attractive on several grounds; for example, electrostatic storage allows high transmitter concentrations inside vesicles without creating osmotic problems. Two recent reports by Parpura and Fernandez (1996), who use atomic force microscopy to examine the mechanical properties of secretory granules, and Marszalek et al. (1996), who measured the diffusivity of serotonin within the granule matrix, add significantly to this picture. The interiors of granules do indeed have mechanical properties similar to an ion-exchange gel, and the diffusion coefficients inside granules are 100–1000 times less than in water. However, most of these experiments have been done at room temperature, and Pihel et al. warn us that the data change considerably at physiological temperatures. Not unexpectedly, the total number of current spikes that reflect exocytosis and secretion into the extracellular space nearly doubles at 37°C compared to 21°C. Furthermore, a representative unitary event is larger at the higher temperature. These effects are reversible suggesting that the observation is not a consequence of increased synthesis of secretory products, and they corroborate a host of earlier results at the tissue level. Although there are a number of ways to interpret these data, they tend to this view: the interiors of granules are gels that hold secretion products and thereby reduce their diffusion even after the granule opens, a mechanism in

Received for publication 3 June 1996 and in final form 12 June 1996.

0006-3495/96/09/1163/02 $2.00

which ionic environment and temperature are bound to play regulatory roles. Putting gels in vesicles raises separate problems. How, for example, are we to picture vesicle membrane recycling? What happens to the gel after release? Are these gels inside small and so-called clear synaptic vesicles? Whatever the answers, the new techniques exploited by the Wightman and Fernandez laboratories will certainly help construct a more accurate picture of chemical secretion. Model vesicle-fusion preparations, like mast cells and chromaffin cells, contain in their granules more than one possible secretion product, including ATP, Ca^{2+}, ascorbate, and other molecules besides traditional transmitters such as catecholamines and serotonin. Beyond that intricacy and its implied opportunity for an enriched program of release, the schematic of release itself is becoming vastly more complex. We know now that the interior of the vesicles is a structured matrix that can regulate the release of its contents and that the mechanism depends profoundly on temperature. Thus the relationship between fusion of vesicle-plasma membranes and the actual release of the secretions is far from obvious. Furthermore, because mast and chromaffin cells behave essentially alike, it is tempting to speculate that the new findings are general. The complexity of fusion and release is only starting to be understood in these relatively simple systems. Even so these new discoveries have already begun to influence the notion of chemical communication in the central and peripheral nervous system, where the mechanisms are inherently more difficult to study.

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