Fusion Gains Independence

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Elevated intracellular Ca^{2+} concentrations [Ca^{2+}] appear to be a rather universal trigger of massive membrane capacitance increases, presumably reflecting exocytosis of tiny vesicles (Borgonovo et al., 2002). The [Ca^{2+}] required to stimulate this response is very high. In this issue Yaradanakul et al. (see p. 29) report experiments with baby hamster kidney (BHK) cells expressing the Na^+/Ca^{2+} exchanger NCX1. In whole-cell patch clamp experiments, using an intracellular solution with high (40 mM) Na^+ concentration and a Na^+-free extracellular solution, switching extracellular [Ca^{2+}] from very low (in the presence of 0.5 mM EGTA) to 2 mM is a new trick that makes it work. The massive Ca^{2+}influx that is produced (mediated bv the Na^+/Ca^{2+} exchanger running in reverse) raises intracellular free [Ca^{2+}] to ~200 µM, which triggers a pronounced capacitance increase. The response is only partially inhibited when ATP is replaced by the nonhydrolyzable analogue AMPPNP.

A Ca^{2+}-dependent capacitance increase, distinct from secretory granule exocytosis, was first discovered in rat peritoneal mast cells (Almers and Neher, 1987) and was subsequently reported to occur in many other cell types (Lindau et al., 1993; Coorssen et al., 1996; Oberhauser et al., 1996; Xu et al., 1998; Borgonovo et al., 2002). The very high [Ca^{2+}] increase required to induce the response in BHK cells agrees with the previously reported requirement for intracellular [Ca^{2+}] exceeding 100 µM to induce corresponding capacitance increases in many other cells—though the response in mast cells was apparent already at ~3 µM free intracellular [Ca^{2+}] (Almers and Neher, 1987).

Phosphoinositides have for many years been implicated to play a significant role in regulated exocytosis (Eberhard et al., 1990; Hay et al., 1995; Martin, 2001; Wenk and De Camilli, 2004). Yaradankul et al. (2008) present detailed studies on the role of phosphoinositides in the response that is triggered by high [Ca^{2+}] in BHK cells. The Ca^{2+} influx activates $PI(4,5)P_2$ breakdown but phosphoinositide metabolism turns out to be neither sufficient nor necessary for the membrane-fusion response. Activation of $PI(4,5)P_2$ breakdown in the absence of a sufficiently high [Ca^{2+}] increase does not stimulate fusion and PLC inhibitors as well as peptides binding $PI(4,5)P_2$ do not interfere with the activation of the Ca^{2+} influx–induced fusion response. These results indicate that the regulation of this fusion response is quite different from what has been reported for hormone release from neuroendocrine cells, where $PI(4,5)P_2$ appears to have a role in the priming as well as the fusion (Eberhard et al., 1990; Hay et al., 1995; Martin, 2001).

What membrane compartment(s) could cause the observed massive capacitance changes? The usual assumption is that these changes reflect an increase in membrane area due to fusion of a large number of small vesicles that are not resolved as individual capacitance steps. Alternatively, for a membrane capacitance increase the plasma membrane thickness would have to decrease or its effective dielectric constant would have to increase dramatically. At present there is no evidence that such changes could occur on the required scale. The size of the response, however, could also not be explained by the vesicle numbers seen in electron micrographs (Yaradanakul et al., 2008). However, in thin sections such small vesicles might be lost, and a mechanism involving vesicle fusion still appears the most likely. Indeed, previous work identified the protein desmoyokin-AHNAK as a marker of the vesicles underlying this exocytotic response and which were named enlargosomes (Borgonovo et al., 2002).

In chromaffin cells the fusion of microvesicles that is not associated with catecholamine release is, in contrast to chromaffin granule exocytosis, not sensitive to tetanus toxin (TeTx) as well as Botulinum neurotoxins E, D, A, and C1 (Xu et al., 1998). Fusion of enlargosomes in PC12 cells is also TeTx insensitive (Kasai et al., 1999; Borgonovo et al., 2002). It would be interesting to explore if the response is also toxin insensitive in BHK cells. If that were the case, exocytosis of enlargosomes could be independent of phosphoinositide turnover.

In the second paper on the pair of articles, Wang and Hilgemann (see p. 51) extend these studies to the rat basophilic leukemia (RBL) mast cell line. Following serotonin loading, the size of secretory granules increases dramatically in RBL cells (Williams et al., 1999). Exocytosis was stimulated by addition of the Ca^{2+} ionophore A23187. Extending the recently developed method of patch amperometry (Albillos et al., 1997; Dernick et al., 2005) to giant patches, the authors demonstrate that large capacitance steps, reflecting fusion

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Abbreviations used in this paper: BHK, baby hamster kidney; RBL, rat basophilic leukemia; TeTx, tetanus toxin.

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of these granules with the plasma membrane, are associated with amperometrically detected serotonin release. In addition to these discrete capacitance steps, capacitance changes that cannot be resolved as discrete steps are observed—and which are not associated with serotonin release, consistent with previous observations in peritoneal mast cells (Oberhauser et al., 1996).

The authors then proceed to explore the underlying mechanism(s) in excised giant patch capacitance measurements, which were performed on cells pretreated with latrunculin A to disrupt the cytoskeleton. Occasionally, but not reproducibly, exocytosis of large secretory granules associated with serotonin release was recorded by patch amperometry also in this configuration. The basis for the variability most likely is that when the patch is excised, the giant granules that are formed by serotonin loading do not stay docked to the cytoplasmic face of the giant patch but are left behind in the cell, tethered to the cytoskeleton.

In giant excised-patch recordings, application of high $[Ca^{2+}]$ from a puffer pipette stimulates massive exocytosis. These experiments were performed on patches from cells that were not preloaded with serotonin. In these cells secretory granules containing mediators such as histamine or hexosaminidase exist but they are small. It is therefore uncertain how much of the response is due to secretory granules and how much is due to microvesicles distinct from the secretory granules.

Surprisingly, the authors find almost complete inhibition of the capacitance response following TeTx treatment. Because rat synaptobrevin-1 is not toxin sensitive (Schiavo et al., 2000), the result suggests that the response is mediated by fusion of vesicles containing synaptobrevin-2 or cellubrevin. This is important because, though exocytosis of mast cell secretory granules is thought not to involve TeTx-sensitive synaptobrevin-2 or cellubrevin, recent experiments suggest that different subpopulations of vesicles undergoing regulated exocytosis in mast cells may involve different VAMP family members (Puri and Roche, 2008). The results of Wang and Hilgemann (2008) suggest that, in contrast to other cell types, exocytosis of RBL vesicles that fuse in response to high intracellular $[Ca^{2+}]$ is mediated by a TeTx-sensitive VAMP family member.

As for the BHK cells, vesicle fusion in RBL cells appears to be independent of phosphoinositide metabolism. However, experiments with wortmannin/adenosine, which inhibits PI(3) and PI(4) kinases, point to a possible role in priming. Using a technique that inflates cells, such that the plasma membrane detaches from the vesicles bound to the cytoskeleton (Solsona et al., 1998), Wang and Hilgemann further show that the recovery of the capacitance response after reshrinking the cells is markedly reduced following wortmannin/adenosine treatment, suggesting that phosphoinositdes may play a role in priming of these vesicles after making contact with the plasma membrane, consistent with the expected role of PI(4,5)P2 in the priming of secretory vesicles in neuroendocrine cells (Hay et al., 1995; Gong et al., 2005; Milosevic et al., 2005). It still remains to be determined what vesicle type gives rise to the large capacitance changes stimulated by $[Ca^{2+}]$ in the hundreds of micromolar range, and if these are at all similar for all the different cell types. The differential TeTx sensitivity in different cell types suggests a quite complex picture.

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