Elevated intracellular Ca\(^{2+}\) concentrations \([\text{Ca}^{2+}]\) appear to be a rather universal trigger of massive membrane capacitance increases, presumably reflecting exocytosis of tiny vesicles (Borgonovo et al., 2002). The \([\text{Ca}^{2+}]\) 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 \([\text{Ca}^{2+}]\) 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 by the Na\(^{+}\)/Ca\(^{2+}\) exchanger running in reverse) raises intracellular free \([\text{Ca}^{2+}]\) to \(\sim 200 \mu\text{M}\), which triggers a pronounced capacitance increase. The response is only partially inhibited when ATP is replaced by the nonhydrolyzable analogue AMPNP.

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 \([\text{Ca}^{2+}]\) increase required to induce the response in BHK cells agrees with the previously reported requirement for intracellular \([\text{Ca}^{2+}]\) exceeding 100 \(\mu\text{M}\) to induce corresponding capacitance increases in many other cells—though the response in mast cells was apparent already at \(\sim 3 \mu\text{M}\) free intracellular \([\text{Ca}^{2+}]\) (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). Yaradanakul et al. (2008) present detailed studies on the role of phosphoinositides in the response that is triggered by high \([\text{Ca}^{2+}]\) 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 \([\text{Ca}^{2+}]\) 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.
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 \([\text{Ca}^{2+}]\) from a puffer pipette stimulates massive exocytosis. However, experiments with wortmannin/adenosine, a TeTx-sensitive VAMP family member.


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**REFERENCES**


