Increased Ca^{2+} buffering enhances Ca^{2+}-dependent process

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The title for this Perspectives article must have a misprint – surely, it should say, 'Increased Ca^{2+} buffering *diminishes* Ca^{2+}-dependent process'. In fact, the title is correct, as shown in the startling results reported in a remarkable study appearing in this issue of *The Journal of Physiology* (Rozov *et al.* 2001). The venerable Neher and Sakmann team has rejoined forces, combining the talents of the two Max-Planck-Institutes in Göttingen and Heidelberg to analyse totally counterintuitive results: perfusing presynaptic neurons with exogenous BAPTA can enhance synaptic facilitation.

In facilitation, successive action potentials (APs) release increasing amounts of transmitter. Substantial evidence attributes facilitation to the lingering effect of a small elevation of presynaptic intracellular calcium concentration ($[Ca^{2+}]_i$), or 'residual calcium' as it is often called (Zucker, 1999). For example, the presynaptic introduction of a Ca^{2+} buffer usually reduces facilitation, presumably by chelating residual Ca^{2+}. Rozov *et al.* (2001) found that EGTA had this familiar effect in synapses from layer 2/3 pyramidal cells in somatosensory cortex of young rats onto bitufted interneurons. Amazingly, introduction of BAPTA had just the opposite effect, actually increasing facilitation at concentrations up to 1 mM. How can extra Ca^{2+} buffering enhance a Ca^{2+}-dependent process?

A simple possibility is that these synapses normally show a net facilitation, but that a mixture of facilitation and depression is really present, with depression due to depletion of a readily releasable store of transmitter. Then a fast buffer like BAPTA that is relatively effective in reducing transmission in response to the first AP, should relieve depression to reveal enhanced net facilitation. A slow buffer like EGTA, on the other hand, is unable to capture much of the Ca^{2+} triggering release near presynaptic Ca^{2+} channels, and so it reduces residual Ca^{2+} without affecting release in response to the first AP, and without changing the amount of depression. However, the high rate of failures in transmission in response to the first AP argues for a low probability of release and a very modest amount of depletion and subsequent depression. Eliminating the calculated underlying depression cannot account for the increased facilitation.

Another possibility is that the synapses operate at a level near saturation, which could reflect saturated occupancy of the secretory trigger by Ca^{2+} or a limitation imposed by some other rate-limiting step. Then reduction of transmission by BAPTA de-saturates release, permitting facilitation to be fully expressed. However, the dependence of release on external calcium concentration ($[Ca^{2+}]_o$) shows that release is nowhere near saturation for these synapses. Both of these explanations also fail to explain why only modest BAPTA concentrations enhance facilitation.

So what is going on? Rozov *et al.* (2001) propose that BAPTA reduces transmission in response to the first AP by chelating some of the entering Ca^{2+} before it reaches the secretory trigger. At modest BAPTA concentrations only, this captured Ca^{2+} partially saturates BAPTA, and less BAPTA is available to buffer Ca^{2+} entering in the next AP. EGTA should be much less subject to such saturation. Two experiments help to confirm this partial saturation model of 'pseudofacilitation' caused by BAPTA. (1) Combining EGTA with BAPTA fails to reduce pseudofacilitation, because EGTA cannot 'steal' Ca^{2+} from the high-affinity BAPTA in the 100 ms interval between APs. (2) Raising $[Ca^{2+}]_o$ enhances pseudofacilitation, by increasing the amount of BAPTA saturation in the first AP. These remarkable results and their interpretation are in line with theoretical studies suggesting that synaptic facilitation could arise from such a saturable buffer (Klingauf & Neher, 1997; Neher, 1998). This suggestion has been largely ignored, perhaps because complex mathematical arguments and simulations were unpersuasive in the absence of experimental data. The suggestion can be ignored no longer.

So now, for the million-dollar question – does normal synaptic facilitation work this way? The results of Rozov *et al.* (2001) indicate that, at least in bitufted cells, the answer is no. Unlike pseudofacilitation, natural facilitation is blocked by EGTA, and is reduced on raising $[Ca^{2+}]_o$. Finally, pseudofacilitation requires that much of the endogenous buffer has high Ca^{2+} affinity, but the strong reduction in transmission by even 0.1 mM BAPTA argues against the presence of such a high-affinity endogenous buffer.

So, how *does* natural facilitation work? This remains somewhat of a mystery. Rozov *et al.* (2001) consider the usual model that facilitation is due to an elevated $[Ca^{2+}]_i$ at the release trigger in a facilitated AP. They calculate that a 25–40 % increase in peak $[Ca^{2+}]_i$ is required. This is unlikely to occur by simple linear summation of a constant Ca^{2+} influx with residual Ca^{2+} from the first AP, because it requires that the residual $[Ca^{2+}]_i$ remains at 1/3 its peak in active zones 100 ms after an AP. Numerous studies calculating the diffusion of Ca^{2+} from active zones indicate that this is exceedingly unlikely, and even the relatively low peak $[Ca^{2+}]_i$ of 10–25 μM calculated to trigger secretion by APs at the calyx of Held (Bollmann *et al.* 2000; Schneggenburger & Neher, 2000) is almost certainly more than 3 times the residual $[Ca^{2+}]_i$ 100 ms after a single AP. Although supralinear summation of Ca^{2+} influx with residual Ca^{2+} is possible (Neher, 1998), this requires a highly saturable endogenous buffer that appears not to be present at pyramidal cell synapses. Facilitation arising from slow dissociation of Ca^{2+} from a separate facilitation complex also is inconsistent with the greater efficacy of EGTA over BAPTA in blocking facilitation. One possibility that has not been ruled out is that facilitation is caused by residual Ca^{2+} acting at a rapidly equilibrating site of moderate affinity some distance from Ca^{2+} channel mouths (Tang *et al.* 2000).

The paper by Rozov *et al.* (2001) is a gold mine of other fascinating results. Pyramidal synapses onto multipolar interneurons in comparison to those onto bitufted interneurons are stronger, show depression rather than facilitation, have an apparently higher Ca^{2+} affinity measured by release dependence on $[Ca^{2+}]_o$, release with shorter synaptic delay, and are less sensitive to exogenous Ca^{2+} buffers. All these results can be explained by a shorter distance of Ca^{2+} channels from docked vesicles in multipolar cell synapses. This paper will occupy the attention of synaptic physiologists for some time, and offers many pleasures and rewards to the careful reader.

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