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How the ‘slow’ Ca^{2+} buffer parvalbumin affects transmitter release in nanodomain-coupling regimes

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Abstract

Parvalbumin is thought to act in a manner similar to EGTA, but how a slow Ca^{2+} buffer affects nanodomain-coupling regimes at GABAergic synapses is unclear. Direct measurements of parvalbumin concentration and paired recordings in rodent hippocampus and cerebellum revealed that parvalbumin affects synaptic dynamics only when expressed at high levels. Modeling suggests that, in high concentrations, parvalbumin may exert BAPTA-like effects, modulating nanodomain coupling via competition with local saturation of endogenous fixed buffers.

Parvalbumin, a major Ca^{2+} -binding protein, is consistently expressed in fast-spiking GABAergic interneurons throughout the hippocampus, cerebellum and neocortex¹. Genetic elimination of parvalbumin causes various alterations in GABAergic synaptic transmission, including an enhancement of synaptic facilitation, a reduction of asynchronous transmitter release and an increase in the power of gamma oscillations^{2–4}. Based on the presence of mixed Ca^{2+} - and Mg^{2+} - binding sites in parvalbumin, it is widely assumed that the buffering properties of parvalbumin are similar to those of the slow buffer EGTA¹. It is unknown how such a slow buffer would affect transmitter release at hippocampal and cerebellar GABAergic synapses, which typically show nanodomain coupling (<100-nm distance) between Ca^{2+} channels and Ca^{2+} sensors^{2,5,6} and are therefore insensitive to EGTA.

To resolve this paradox, we measured the absolute concentration of parvalbumin in GABAergic interneurons using calibrated immunohistochemistry⁷ (Fig. 1, Supplementary Fig. 1 and Supplementary Methods). In each slice, a reference cell (lacking the expression of endogenous parvalbumin) was filled with a defined concentration of recombinant parvalbumin and used for subsequent quantification. Control experiments with two neurons filled with different parvalbumin concentrations in the same slice demonstrated the linearity of the assay (Supplementary Fig. 1e). Analysis of the somatic parvalbumin concentration in hippocampal dentate gyrus basket cells⁵ revealed that the average parvalbumin concentration was low and highly variable (mean parvalbumin concentration = 11.9 ± 1.6 μM , range = 0.8–70.6 μM , coefficient of variation = 0.97 ± 0.10 , 311 cells in 6 slices; Fig. 1a,b,e). Analysis of noninvasively labeled hippocampal basket cells further revealed that the

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mean parvalbumin concentration was almost identical in boutons and somata (fluorescence ratio = 1.01 ± 0.15 , 448 boutons of 3 cells; Supplementary Fig. 2).

In contrast, the somatic parvalbumin concentration in cerebellar basket cells was markedly higher and less variable than in hippocampal basket cells (mean parvalbumin concentration = $563 \pm 66 \mu\text{M}$, range = 55–1,788 μM , coefficient of variation = 0.58 ± 0.02 , 197 cells in 5 slices; Fig. 1c,d,f). Similar differences in parvalbumin concentration between cerebellar and hippocampal basket cells were observed in brain tissue from adult rats (Supplementary Fig. 3).

In the hippocampal dentate gyrus, we found no functional differences between *Pvalb*^{+/+} and *Pvalb*^{-/-} synapses (Supplementary Fig. 4), consistent with the low concentration of parvalbumin (but see ref. 4). In contrast, robust effects were detected in cerebellar GABAergic synapses. Although unitary inhibitory postsynaptic currents (IPSCs) evoked by single presynaptic action potentials were unchanged (Supplementary Fig. 5), we observed a marked difference in synaptic dynamics² (Fig. 1g,h). In *Pvalb*^{+/+} synapses, IPSCs during the train showed a slight facilitation followed by a moderate depression. In contrast, in *Pvalb*^{-/-} synapses, a high degree of facilitation was consistently observed (Fig. 1i). On average, the ratio of the second IPSC to the first IPSC (IPSC₂/IPSC₁) increased from 1.09 ± 0.08 to 1.55 ± 0.12 , whereas IPSC₁₀/IPSC₁ changed from 0.76 ± 0.12 to 1.27 ± 0.17 after genetic elimination of parvalbumin ($P < 0.05$, 9 and 8 pairs, respectively). Similar results were obtained at nearphysiological temperature (Supplementary Fig. 6). These results confirm previous observations in cerebellar basket cell output synapses².

To determine whether residual Ca^{2+} is involved in facilitation in *Pvalb*^{-/-} synapses, we examined the extent of asynchronous release, that is thought to be driven by residual Ca^{2+} (ref. 8). Absolute release rates were quantified by deconvolution (Fig. 1j and Supplementary Fig. 7). However, the ratio of synchronous to asynchronous release at cerebellar basket cell–Purkinje cell synapses was not significantly different between *Pvalb*^{+/+} and *Pvalb*^{-/-} synapses (7.2:1 and 9.8:1, respectively; $P > 0.5$). We next examined whether the effects of chronic elimination of parvalbumin could be rescued by acute application of recombinant parvalbumin (Fig. 1k,l and Supplementary Fig. 8a–c). After loading the presynaptic neuron in *Pvalb*^{-/-} slices with 300 or 500 μM recombinant parvalbumin, IPSC₂/IPSC₁ decreased from 1.33 ± 0.12 to 0.76 ± 0.16 over >13 min of recording time ($P < 0.05$, 6 pairs). Finally, we tested whether the effects of parvalbumin were gene copy number dependent (Supplementary Fig. 8d–f). IPSC₂/IPSC₁ was 1.30 ± 0.09 for *Pvalb*^{+/+} synapses, roughly intermediate between *Pvalb*^{+/+} and *Pvalb*^{-/-} synapses ($P > 0.1$, 6 pairs).

It is widely assumed that the buffering properties of parvalbumin are similar to those of EGTA¹. How can a slow buffer affect transmitter release in nanodomain-coupling regimes at GABAergic synapses?^{2,5,6} Given that direct measurements of Ca^{2+} nanodomains are not possible, we addressed this question using computational modeling. Ca^{2+} diffusion in nanodomains around a Ca^{2+} point source was simulated in the presence of various buffers, using the time-dependent solution to the full set of reaction and diffusion equations^{5,9} (Fig. 2). Notably, the effects of parvalbumin on the Ca^{2+} transient evoked by an action potential in the center and the periphery of the nanodomain were distinct from those of both BAPTA and EGTA. The fast buffer BAPTA reduced both the amplitude and duration of the Ca^{2+} transient and the slow buffer EGTA primarily reduced the residual Ca^{2+} concentration after the action potential, whereas the effects of parvalbumin were intermediate (Fig. 2a,b). Thus, parvalbumin does not exclusively act in a manner similar to EGTA¹, but also may act similarly to BAPTA under physiological conditions. This can be understood from the substantial concentration of free parvalbumin. Under resting conditions (40 nM free Ca^{2+} (ref. 3), 400 μM free Mg^{2+}), 5% of the parvalbumin in our model was free, 73% was bound

to Mg^{2+} and 22% was bound to Ca^{2+} . Furthermore, parvalbumin differed from both exogenous buffers in regeneration properties (Fig. 2c–e). Different from both BAPTA and EGTA (Fig. 2c,d), the concentration of free parvalbumin dropped initially and was slowly, but efficiently, replenished (Fig. 2e). Comparison of the effects of mobile versus fixed variants of parvalbumin revealed that regeneration of free buffer primarily took place from the pool of Mg^{2+} -bound parvalbumin (Supplementary Fig. 9). Thus, the Mg^{2+} binding of parvalbumin implements a mechanism for buffering the concentration of the free buffer, a process we term “metabuffering.”

In microdomain-coupling regimes, facilitation of transmitter release is thought to mainly rely on residual Ca^{2+} (ref. 8). In nanodomain-coupling regimes, however, the relative effect of residual Ca^{2+} is smaller, and facilitation of Ca^{2+} transients by local saturation of fixed buffer may substantially contribute^{9–12}. We therefore modeled nanodomain coupling in the presence of different concentrations of parvalbumin and fixed buffer (Fig. 2f–j)⁵. Following an action potential, the concentration of the free fixed buffer dropped to almost zero near the source and the reduction was long-lasting (Fig. 2f). The concentration of free parvalbumin dropped initially, but was efficiently regenerated in <5 ms (Fig. 2g). To address the differential effects of buffer saturation and regeneration on release, we stimulated two or ten action potentials at 20-ms intervals in various parvalbumin and fixed buffer scenarios (Fig. 2h–j). In the exclusive presence of 100 μM fixed buffer, the Ca^{2+} transient was markedly facilitated (Fig. 2h), especially at the border of the Ca^{2+} nanodomain. In contrast, in the exclusive presence of 1 mM parvalbumin, the second Ca^{2+} transient was almost identical to the first (Fig. 2h). In a mixed buffer scenario, 10 μM to 1 mM parvalbumin reduced the extent of facilitation of the second Ca^{2+} transient in a concentration-dependent manner (Fig. 2h–j). The anti-facilitation effect of parvalbumin was confirmed for different Mg^{2+} concentrations, a tenfold range of concentrations and rate constants of the fixed buffer, and coupling configurations with distributed Ca^{2+} channels (Supplementary Figs. 10–12). Finally, simulations based on a previously established model of transmitter release confirmed that small effects of fixed buffer and parvalbumin on the Ca^{2+} transient were converted into large effects on release, as predicted by the high-power relationship between the two quantities (Supplementary Fig. 13)¹³. Although it does not exclude other mechanisms of facilitation of transmitter release (for example, mediated by residual Ca^{2+}), our model suggests that, in nanodomain-coupling regimes, facilitation may be generated by local saturation of fixed buffer and parvalbumin may regulate synaptic dynamics by shunting local buffer saturation.

We found that the concentration of parvalbumin in cerebellar basket cells was higher than previously thought, approaching the concentration found in skeletal muscle ($\sim 1 \text{ mM}$)¹. Thus, although the fraction of free parvalbumin (that is, parvalbumin not bound to either Mg^{2+} or Ca^{2+}) present under physiological conditions was <10%, the absolute concentration of the free buffer becomes substantial. This has two major consequences. Our work suggests that, first, parvalbumin may not exclusively act similar to EGTA, but also similar to BAPTA under physiological conditions. This explains why parvalbumin affects synaptic transmission in nanodomain-coupling regimes. Second, the Mg^{2+} -bound parvalbumin fraction will not slow the effective Ca^{2+} -binding rate, but instead contributes to buffer regeneration. Thus, Mg^{2+} binding establishes a metabuffering mechanism, maintaining the concentration of free parvalbumin during repetitive activity. Because of these properties, parvalbumin can act as an efficient anti-facilitation factor². It is widely assumed that facilitation of transmitter release is mediated by residual Ca^{2+} , but other mechanisms, such as presynaptic action potential broadening, facilitation of presynaptic Ca^{2+} channels, and facilitation of the presynaptic Ca^{2+} transient by buffer saturation, have also been proposed¹⁴. However, a major role of residual Ca^{2+} at cerebellar GABAergic synapses cannot be easily reconciled with the lack of effects on asynchronous release that we observed and the

previously reported decrease of asynchronous release in *Pvalb*^{-/-} mice³. In contrast, local buffer saturation seems consistent with experimental observations^{10,11}. In this scenario, the free form of parvalbumin competes with saturation of the fixed buffer. The efficacy of parvalbumin is explained by both regeneration from Mg²⁺-bound states and diffusion from the surround of the nanodomain¹⁵ (Supplementary Fig. 14).

Supplementary Material

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References

1. Schwaller B, Meyer M, Schiffmann S. *Cerebellum*. 2002; 1:241–258. [PubMed: 12879963]
2. Caillard O, et al. *Proc. Natl. Acad. Sci. USA*. 2000; 97:13372–13377. [PubMed: 11069288]
3. Collin T, et al. *J. Neurosci.* 2005; 25:96–107. [PubMed: 15634771]
4. Vreugdenhil M, Jefferys JGR, Celio MR, Schwaller B. *J. Neurophysiol.* 2003; 89:1414–1422. [PubMed: 12626620]
5. Bucurenciu I, Kulik A, Schwaller B, Frotscher M, Jonas P. *Neuron*. 2008; 57:536–545. [PubMed: 18304483]
6. Christie JM, Chiu DN, Jahr CE. *Nat. Neurosci.* 2011; 14:62–68. [PubMed: 21170054]
7. Müller A, et al. *J. Neurosci.* 2005; 25:558–565. [PubMed: 15659591]
8. Atluri PP, Regehr WG. *J. Neurosci.* 1996; 16:5661–5671. [PubMed: 8795622]
9. Matveev V, Zucker RS, Sherman A. *Biophys. J.* 2004; 86:2691–2709. [PubMed: 15111389]
10. Rozov A, Burnashev N, Sakmann B, Neher E. *J. Physiol. (Lond.)*. 2001; 531:807–826. [PubMed: 11251060]
11. Felmy F, Neher E, Schneggenburger R. *Neuron*. 2003; 37:801–811. [PubMed: 12628170]
12. Faas GC, Raghavachari S, Lisman JE, Mody I. *Nat. Neurosci.* 2011; 14:301–304. [PubMed: 21258328]
13. Sakaba T. *Neuron*. 2008; 57:406–419. [PubMed: 18255033]
14. Zucker RS, Regehr WG. *Annu. Rev. Physiol.* 2002; 64:355–405. [PubMed: 11826273]
15. Schmidt H, Brown EB, Schwaller B, Eilers J. *Biophys. J.* 2003; 84:2599–2608. [PubMed: 12668468]

Figure 1.

Cell-specific differences in somatic parvalbumin concentration correlate with synapse-specific effects on synaptic dynamics. **(a)** Confocal stack maximum projection of the dentate gyrus and the CA3 region, showing a granule cell filled with biocytin and 30 μ M recombinant parvalbumin and several surrounding cells. **(b)** Expanded views, with the right and bottom panels corresponding to the rectangle in **a**. The left two panels show biocytin labeling, the top right panel shows parvalbumin immunoreactivity and the bottom right panel is an overlay. Scale bars represent 100 μ m (top left) and 25 μ m (bottom left and both right panels). **(c)** Analysis of parvalbumin concentration in cerebellar basket cells. The image is a confocal stack maximum projection of the molecular layer, Purkinje cell layer and granule cell layer of the cerebellum, showing a cell filled with biocytin and 30 μ M recombinant parvalbumin and several surrounding cells. **(d)** Expanded view corresponding to the rectangle in **c**. Top, biocytin labeling. Middle, parvalbumin immunoreactivity. Bottom, overlay. **(e,f)** Histograms of the somatic parvalbumin (PV) concentration in 64 hippocampal basket cells **(e)** and 53 cerebellar basket cells **(f)**, each in a representative slice. Insets, mean parvalbumin concentration and coefficient of variation of 6 and 5 slices, respectively. Error bars indicate s.e.m. **(g,h)** Short-term dynamics during a 50-Hz train of 10 action potentials in paired recordings between basket cells and synaptically connected Purkinje cells in cerebellar slices from *Pvalb*^{+/+} **(g)** and *Pvalb*^{-/-} mice **(h)**. Red traces, presynaptic action potentials evoked by brief current pulses; black traces, average IPSCs. **(i)** Amplitude of the *j*th IPSC in the train measured from the preceding baseline and divided by the amplitude of the first IPSC. Data are from 9 (*Pvalb*^{+/+}, black) and 8 (*Pvalb*^{-/-}, red) pairs, respectively. **(j)** Relative contribution of synchronous release (Syn), asynchronous release during the train (Asyn 1), and asynchronous release after the train (Asyn 2) in *Pvalb*^{+/+} (black) and *Pvalb*^{-/-} synapses (red). 50-Hz trains of ten presynaptic action potentials were used in all experiments. Error bars in **i** and **j** indicate s.e.m. **(k,l)** Rescue of *Pvalb* deletion by acute presynaptic infusion of 500 μ M recombinant parvalbumin (rPV). Shown is a recording from a cerebellar basket cell–Purkinje cell synapse from a *Pvalb*^{-/-} mouse 0–6 min after break in **(k)** and 35–41 min after break in **(l)**.

Figure 2.

Parvalbumin may have fast effects and regulate synaptic dynamics by shunting of local buffer saturation. (a,b) Ca^{2+} transients following a single action potential 20 nm (a) and 200 nm (b), respectively, from the point source. Inset in a shows expansion of the peak region. Concentrations: BAPTA (100 μM), EGTA (100 μM) and parvalbumin (50 μM protein concentration, corresponding to 100 μM buffer concentration, as parvalbumin has two Ca^{2+} -binding sites). Illustrations on top schematically depict the binding scheme of parvalbumin and the binding schemes for BAPTA and EGTA (buffer). (c–e) Parvalbumin has specific regeneration properties. (f,g) Spatiotemporal profile of concentration of free fixed buffer (FB, f) and parvalbumin (g) in a mixed fixed buffer and parvalbumin scenario following a single action potential (50 μM parvalbumin protein concentration; 100 μM fixed buffer). (h) Facilitation of Ca^{2+} transient at 20, 50 and 200 nm from the Ca^{2+} channels. Two action potentials were simulated at 20-ms intervals. Ca^{2+} transients are shown normalized to the peak of the first Ca^{2+} transient and horizontal lines indicate facilitation. Bottom right, expansion of the peak region of the second Ca^{2+} transient at the three distances. (i) Multiple-pulse facilitation (MPF) of the Ca^{2+} transient 50 nm from the Ca^{2+} channels, plotted against action potential number. (j) MPF of the Ca^{2+} transient for the tenth action potential plotted against source-sensor distance. Insets in i and j show the corresponding MPF of transmitter release, predicted from a power relation. Longer line segments indicate higher parvalbumin concentrations.