A Simple Depletion Model of the Readily Releasable Pool of Synaptic Vesicles Cannot Account for Paired-Pulse Depression

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Sullivan JM. A simple depletion model of the readily releasable pool of synaptic vesicles cannot account for paired-pulse depression. J Neurophysiol 97: 948–950, 2007. First published November 1, 2006; doi:10.1152/jn.00554.2006. Paired-pulse depression (PPD) is a form of short-term plasticity that plays a central role in processing of synaptic activity and is manifest as a decrease in the size of the response to the second of two closely timed stimuli. Despite mounting evidence to the contrary, PPD is still commonly thought to reflect depletion of the pool of synaptic vesicles available for release in response to the second stimulus. Here it is shown that PPD cannot be accounted for by depletion at excitatory synapses made by hippocampal neurons because PPD is unaffected by changes in the fraction of the readily releasable pool (RRP) released by the first of a pair of pulses.

INTRODUCTION

In its simplest form, the depletion model of depression proposes that each action potential triggers the release of a constant fraction of the pool of readily releasable vesicles (RRP) (Betz 1970; Liley and North 1953; Zucker and Regehr 2002). According to this model, when two closely timed stimuli are delivered, the response to the second stimulus will be smaller than the first (depressed) because the RRP is transiently reduced by the number of vesicles released in response to the first stimulus; recovery from depression proceeds as the depleted RRP is refilled over several seconds. Although appealing, this simple model is unlikely to account for the depression that is observed at excitatory hippocampal synapses because back-of-the-envelope calculations incorporating an average release probability per synapse of ~0.2 (in 2 mM external calcium; Murthy et al. 1997) and an average RRP size of ~6–10 vesicles (Harris and Sultan 1995; Murthy et al. 2001; Schikorski and Stevens 1997) underestimate experimentally observed values of PPD (Mennerick and Zorumski 1995; Sippy et al. 2003). Elegant studies at the calyx of Held have implicated heterogeneity of release probability for vesicles within the releasable pool (Sakaba and Neher 2001; Wu and Borst 1999) and calcium-dependent inhibition of presynaptic calcium currents (Xu and Wu 2005) as major mechanisms underlying depression at this giant CNS synapse, but leave open the possibility that depletion could account for depression at smaller hippocampal synapses. Here it is shown that simple depletion cannot account for the paired-pulse depression (PPD) observed at these hippocampal synapses.

METHODS

Culture preparation

Rat hippocampal neurons isolated from the CA1–CA3 regions were cultured on microislands. Neurons were plated onto a feeder layer of astrocytes that had been laid down 1–7 days earlier and grown in medium containing 10% horse serum. Neurons were grown without mitotic inhibitors and used for recordings after 9 days in culture.

Electrophysiology

When a single neuron is grown on a small island of permissive substrate, it forms synapses (autapses) onto itself. All experiments were performed on isolated autaptic neurons after AM-EGTA treatment (300 µM for 10 min) to buffer intraterminal calcium, thereby removing facilitation. Whole cell voltage-clamp recordings were carried out using Axopatch 200B amplifier (Axon Instruments, Burlingame, CA). The extracellular solution contained (in mM) 119 NaCl, 5 KCl, 1.0 CaCl₂, 3.0 MgCl₂, 30 glucose, and 20 HEPES. For those experiments where the extracellular calcium concentration was altered, the magnesium concentration was adjusted to maintain a constant concentration of divalent cations. Recording pipettes of ~2-5 MΩ were filled with (in mM) 121.5 KGlucanate, 17.5 KCl, 9 NaCl, 1 MgCl₂, 10 HEPES, 0.2 EGTA, 2 MgATP, and 0.5 LiGTP. Access resistance was monitored, and only cells with stable access resistance (<20% deviation from initial value) were included in the data analysis. Data were acquired at a rate of 2 kHz. The membrane potential was held at ~60 mV, and pairs of excitatory postsynaptic currents (EPSCs) were evoked by triggering unclamped action currents with 1-ms depolarizing steps separated by a 45-ms interpulse interval. PPD was monitored using the paired-pulse ratio (PPR). To calculate the PPR, the peak amplitude of the response to the second pulse was divided by the peak amplitude of the response to the first pulse. To calculate the PPR at rest for the data in Fig. 1, no more than six PPR values collected at rest were averaged from each cell. For all other data points, the PPR at the indicated time after depletion of the RRP for each cell was calculated from a single pair of responses. A 1-min interval separated the stimulation sets (paired pulses—pool depletion—paired pulses).

Hypertonic solution application

The RRP of vesicles was completely depleted by a 4- to 5-s application of hypertonic solution (normal extracellular solution without divalent cations plus 500 mM sucrose) using a picospritzer-controlled puffer pipette. A vacuum pipette cleared the hypertonic solution rapidly. The hypertonic solution was applied over the entire
island on which the autaptic neuron was located to ensure that the same population of synapses was activated every time the solution was applied and that this same population was activated by electrical stimulation at the neuronal cell body.

RESULTS

To probe the relationship between depletion and depression, PPD was measured while the size of the RRP was varied. To isolate mechanisms underlying depression, experiments were performed using cultured hippocampal neurons that had been treated with the membrane-permeable calcium chelator AM-EGTA to block facilitation: note that fast calcium-dependent processes will not be blocked with this relatively slow chelator. After EGTA-loading, there was no significant effect of RRP depletion on the ratio of EPSCs elicited by pairs of electrical stimuli (separated by 45 ms) that were presented at various times after the RRP was emptied by a 5-s-long superfusion of hypertonic solution. Although the 1st pair of stimuli were delivered to elicit 2 more synaptic responses (EPSC1a and EPSC2a), the 1st response (EPSC1a) was not significantly different from the 2nd response (EPSC2a), either in the same 1 mM external Ca$^{2+}$ or in AM-EGTA treatment. Action currents here and elsewhere have been blanked for clarity. At 8.7 s after the end of hypertonic solution application, synaptic responses were reduced to about one third of their original size, presumably because of depletion of RRP, but PPR showed little change (0.81); scale bar: 250 pA, 15 ms. Graph shows pooled results plotting PPRb (EPSC2b/EPSC1b) against time after depletion of RRP by application of hypertonic solution (n = 7) or delivery of a train of action potentials (n = 7); linear regression lines do not deviate significantly from 0.

FIG. 1. Paired-pulse depression (PPD) is not affected by the size of the pool of readily releasable vesicles (RRP). Top: schematic (not to scale) of experimental protocol: a pair of brief depolarizing stimuli were delivered 45 ms apart to elicit 2 synaptic responses at rest [excitatory postsynaptic current (EPSC1) and EPSC2]. The 1st pair of stimuli was followed by a 5-s application of a Ca$^{2+}$-free external solution made hypertonic by the addition of 0.5 M sucrose to completely deplete the RRP. At varying delays after depletion of RRP, a 2nd pair of stimuli was delivered to elicit 2 more synaptic responses (EPSC1b and EPSC2b). Bottom: typical responses to this stimulus paradigm: on the left, autaptic response at rest, with a paired-pulse ratio (PPR) of 0.78 in 1 mM external Ca$^{2+}$ after AM-EGTA treatment. Action currents here and elsewhere have been blanked for clarity. At 8.7 s after the end of hypertonic solution application, synaptic responses were reduced to about one third of their original size, presumably because of depletion of RRP, but PPR showed little change (0.81); scale bar: 250 pA, 15 ms. Graph shows pooled results plotting PPRb (EPSC2b/EPSC1b) against time after depletion of RRP by application of hypertonic solution (n = 7) or delivery of a train of action potentials (n = 7); linear regression lines do not deviate significantly from 0.

FIG. 2. Even when a pair of stimuli releases a large fraction of the RRP, PPD is not enhanced. A: top left: schematic (not to scale) of experimental paradigm used for these experiments. First, a pair of stimuli are delivered 45 ms apart to elicit 2 synaptic responses at rest (EPSC1a and EPSC2a), followed by a 5-s application of hypertonic solution to completely deplete the RRP. At 6.2 s after depletion of RRP, a 2nd pair of stimuli is delivered to elicit 2 more synaptic responses (EPSC1b and EPSC2b), either in the same 1 mM external Ca$^{2+}$ or in elevated (2.5 mM) external Ca$^{2+}$. Bottom left: response of an AM-EGTA–treated cell to a pair of stimuli in 2.5 mM Ca$^{2+}$ at rest. Top right: response of the same cell to a pair of stimuli in 1 mM Ca$^{2+}$ delivered 6.2 s after application of hypertonic solution. Bottom right: response to a pair of stimuli in 2.5 mM Ca$^{2+}$ delivered 6.2 s after application of hypertonic solution. Although the 1st response to the 2nd pair of stimuli is much smaller in 1 mM Ca$^{2+}$ (EPSC1b $\ll$ EPSC1a), the 1st response to the 2nd pair of stimuli is about the same size in 2.5 mM Ca$^{2+}$ (EPSC1b $\approx$ EPSC1a). Scale bar: 250 pA, 15 ms. Despite large difference in amplitude, there is little difference in PPR of these responses. Data from 5 cells are plotted in B showing relative amplitude of the 1st responses (EPSC1a/EPSC1b) to the 2nd pair of stimuli in 1 and 2.5 mM Ca$^{2+}$. C: PPR in these cells is not altered when a much larger fraction of RRP is depleted by the 1st of a pair of stimuli.
5) at rest in 1 mM Ca^{2+}; 6.2 s after the RRP was emptied, the amplitude of the first response to the second pair of stimuli was one fifth the size of the first response to the first pair (0.20 ± 0.12) when external Ca^{2+} remained at 1 mM. When external Ca^{2+} was raised by rapid application of solution containing 2.5 mM Ca^{2+}, the amplitude of the first response to the second pair of stimuli was approximately the same size as the first response evoked by the first pair (1.00 ± 0.25). Contrary to the expectations of the depletion model, under these conditions, where a much larger fraction of the well-depleted RRP was released because of the increase in external Ca^{2+}, PPD remained identical to that observed at rest in 1 mM Ca^{2+} (0.87 ± 0.05 after depletion in 2.5 mM Ca^{2+}). Similar results were obtained when trains of action potentials were used to deplete the pool (data not shown). The lack of effect on PPD after changing external Ca^{2+} was not specific to synapses with depleted pools, because there was also no difference in PPD at rest when external Ca^{2+} was shifted between 1 and 2.5 mM Ca (0.88 ± 0.06 and 0.83 ± 0.05, respectively; n = 6). The insensitivity of PPD to changes in external calcium concentration may seem surprising in light of previous work (Zucker and Regehr 2000), but prior studies examining the relationship between the PPR and external calcium (and release probability in general) have not typically been performed under conditions that block facilitation; the simplest explanation is that paired-pulse facilitation is sensitive to external calcium concentration, but PPD is not. Taken together, these results directly refute the simple depletion model by showing that, even when a significantly greater fraction of the RRP is released by the first stimulus of a pair, there is no enhancement of the depression of release in response to the second stimulus, relative to the amount of depression seen at rest.

**DISCUSSION**

Although depletion almost certainly plays a key role in the reduction of response size during a train of action potentials, the data presented here show that a simple pool depletion model cannot account for PPD at hippocampal excitatory synapses. A number of mechanisms remain plausible candidates for inducing PPD at this mammalian CNS synapse, including heterogeneity of release probability between vesicles (Moulder and Mennerick 2005; Sakaba and Neher 2001; Wu and Borst 1999) and use-dependent inhibition of release machinery (Hsu et al. 1996; Waldeck et al. 2000), but the one most consistent with this study is the calcium-dependent inhibition of the presynaptic calcium currents responsible for triggering release (Xu and Wu 2005; see also Li et al. 2006), particularly because this inhibition seems to saturate with small amounts of calcium entry and would therefore be relatively insensitive to either the presence of a slow calcium buffer, such as the AM-EGTA used here, or changes in external calcium concentration.

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