

Vesicular Calcium Transport Shapes Rapid Acetylcholine Secretion

J. Miguel Cordeiro,^{*,1,2} Yves Dunant,² and Paula P. Gonçalves¹

¹CESAM, Departamento Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal;
and ²Département de Neurosciences, CMU, 1211 Geneva 4, Switzerland

Introduction

Rapid secretion relies on the occurrence of spike-like Ca^{2+} transients in active zones (Llinás et al., 1992; Yazejian et al., 2000; Dunant and Bloc, 2003). Presynaptic Ca^{2+} nanodomains are to be restricted both in time and in space as to assure rapid onset and termination of transmitter release (Llinás et al., 1992; Pozzan et al., 1994; Yazejian et al., 2000; Dunant and Bloc, 2003). A very fast Ca^{2+} -buffering mechanism should allow Ca^{2+} rise above $\sim 100 \mu\text{M}$ for less than $\sim 250 \mu\text{s}$ and then rapid reduction of Ca^{2+} to subthreshold levels of release (Llinás et al., 1992; Pozzan et al., 1994; Yazejian et al., 2000; Dunant and Bloc, 2003). Swift Ca^{2+} clearance by vesicular $\text{Ca}^{2+}/\text{H}^{+}$ antiport as a low-affinity, high-capacity extrusion mechanism was postulated in the past (Pozzan et al., 1994; Dunant and Bloc, 2003). We demonstrated pH gradient (ΔpH)-dependent Ca^{2+} uptake by mammalian brain synaptic vesicles (Gonçalves et al., 1998, 2000). Moreover, this antiport activity is effective at $[\text{Ca}^{2+}]$ ranging from ~ 100 to $800 \mu\text{M}$ (max. at $\sim 500 \mu\text{M}$) (Gonçalves et al., 1998, 2000). We now show that the time course of acetylcholine (ACh) secretion in *Torpedo* neuro-electrocytic synapse is modified by bafilomycin A1 (baf.), which compromises antiport activity. Along with this mechanism, synaptic vesicles also have a P-type Ca^{2+} ATPase, exhibiting half-maximal activation for $0.6 \mu\text{M}$ Ca^{2+} (Gonçalves et al., 2000). Here, we demonstrate the role of P-type Ca^{2+} ATPase in

preventing desensitization of the release mechanism by inhibiting it with orthovanadate.

Results and Discussion

Rapid Ca^{2+} sequestration operated by vesicular $\text{Ca}^{2+}/\text{H}^{+}$ antiport was disrupted by using the specific V-type H^{+} -ATPase inhibitor baf. This abolishes the ΔpH of synaptic vesicles that energizes the antiport and finally results in prolongation of the postsynaptic current from 2–4 ms up to >10 ms, by persistence of ACh release (Fig. 1A–I, A–II). Simultaneous measurement of $[\text{C}^{14}]\text{ACh}$ release confirmed the presynaptic nature of this phenomenon. Moreover, drug washout returned values to control levels (Fig. 1B–I, B–II). Accordingly, baf. increased ACh release from electric organ synaptosomes, elicited by either veratridine or KCl depolarization, followed by Ca^{2+} addition. Conversely, when synaptosomes were exposed longer than 10 min to baf. in the presence of $[\text{Ca}^{2+}]_{\text{out}} = 3.5 \text{ mM}$ prior to depolarization, desensitization of release occurred (not illustrated). Disrupting Ca^{2+} ATPase with $10 \mu\text{M}$ orthovanadate resulted in slow (hours) failure of transmission (Fig. 2) by a Ca^{2+} -dependent, presynaptic effect. $50 \mu\text{M}$ of BAPTA-AM, perfused in the presence of orthovanadate, quickly recuperated transmission (Fig. 2A), whereas orthovanadate washout was less efficient (Fig. 2B).

Phasic Ca^{2+} sequestration by vesicular $\text{Ca}^{2+}/\text{H}^{+}$ antiport activity shapes the time course of secretion at microsecond range. The antiport also participates

*Author to whom all correspondence and reprint requests should be addressed. E-mail: mcordeiro@bio.ua.pt

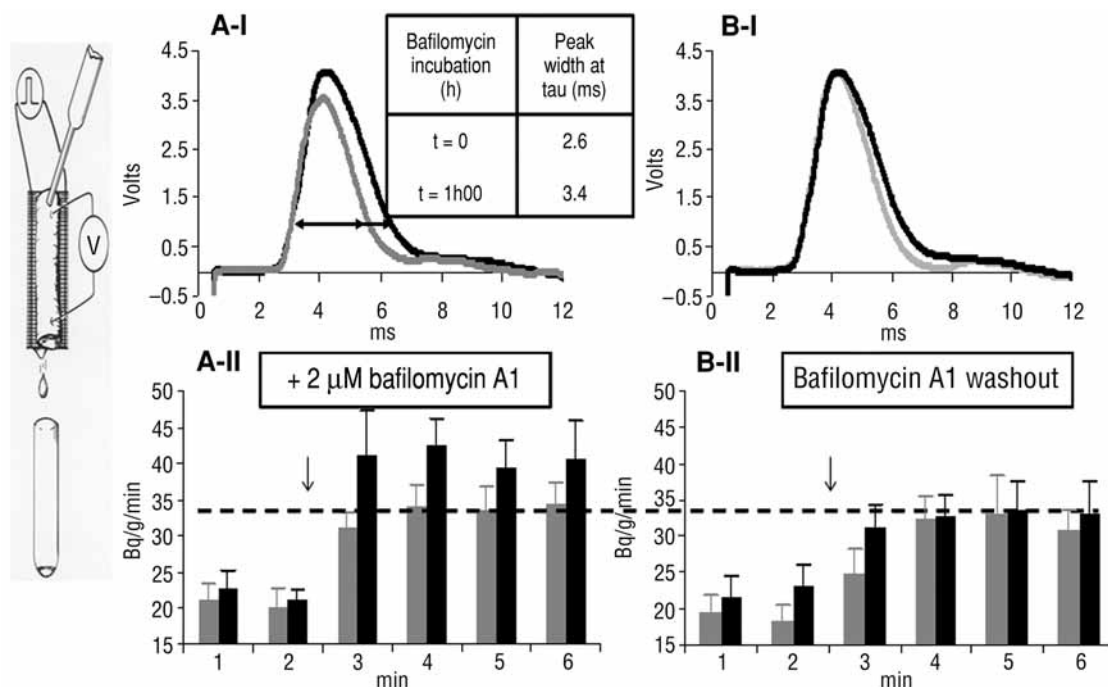


Fig. 1. *Torpedo* electric organ stacks of electrocytes (prisms) were labeled overnight with ^{14}C -labeled acetate (Dunant et al., 1980). Prisms were perfused with 2 μM baf. for 1 h prior to stimulation (A, black bars and trace). Control prisms are shown in gray. [^{14}C]ACh diffused from tissue was collected before and after stimulus (\downarrow , A-II). Electrocyte responses were measured (A-I) at the same time. The same parameters were assessed after 1 h drug washout (B-I, B-II). The black trace in B-I shows baf. effect as reference vs washout in gray.

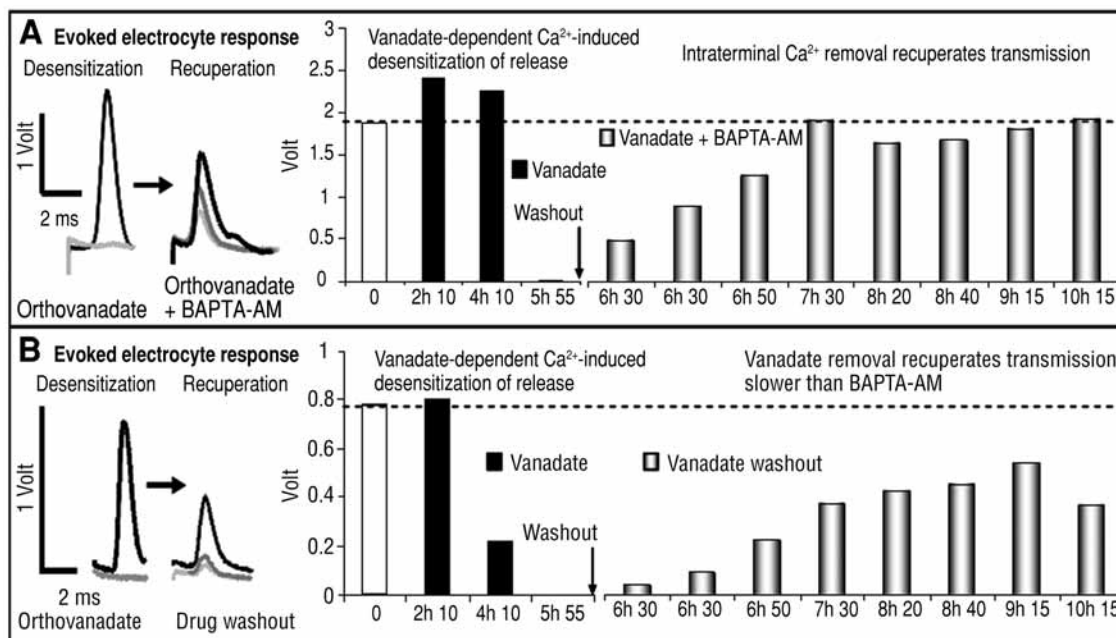


Fig. 2. *Torpedo* prisms were perfused continuously with marine solution containing 10 μM Na^+ -orthovanadate (black bars). Evoked electrophysiological responses were recorded for several hours. Complete desensitization of release mechanism (after 5 h and 55 min) is shown in traces, followed by rapid recuperation (A), after adding 50 μM BAPTA-AM (55 min) to medium or slow recuperation (B), by washing out orthovanadate. Bars show discharge amplitude.

in late Ca²⁺ homeostasis and extrusion (Dunant, this issue), working with Ca²⁺ ATPase in housekeeping tasks.

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