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ORIGINAL ARTICLE

Vesicular Calcium Transport Shapes Rapid Acetylcholine Secretion

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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 ~100 µ*M* for less than ~250 µs 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 Ca^{2+}/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 $[Ca^{2+}]$ ranging from ~100 to 800 µ*M* (max. at ~500 µ*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 µM 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 Ca^{2+}/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 $[^{14}C]$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 $[Ca^{2+}]_{out}$ = 3.5 m*M* prior to depolarization, desensitization of release occurred (not illustrated). Disrupting Ca^{2+} ATPase with 10 µ*M* orthovanadate resulted in slow (hours) failure of transmission (Fig. 2) by a Ca^{2+}-dependent, presynaptic effect. 50 µ*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 Ca^{2+}/H^+ antiport activity shapes the time course of secretion at microsecond range. The antiport also participates

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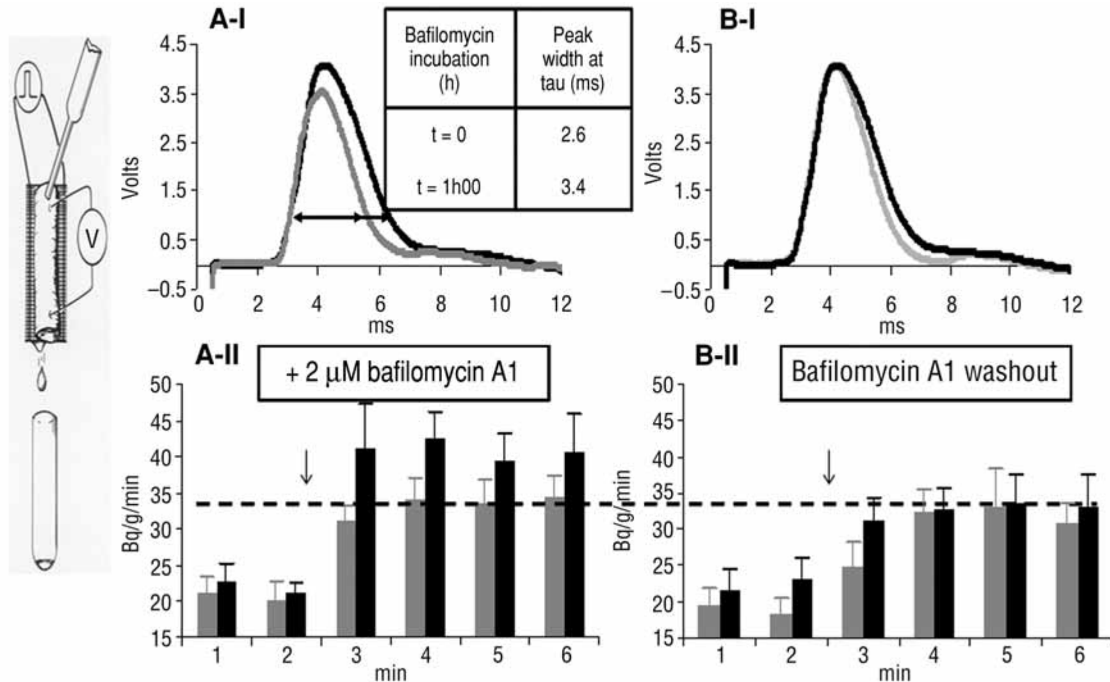

Fig. 1. *Torpedo* electric organ stacks of electrocytes (prisms) were labeled overnight with ^{14}C-labeled acetate (Dunant et al., 1980). Prisms were perfused with 2 μ*M* baf. for 1 h prior to stimulation (**A,** black bars and trace). Control prisms are shown in gray. [^{14}C]ACh diffused from tissue was collected before and after stimulus **(**↓, **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.

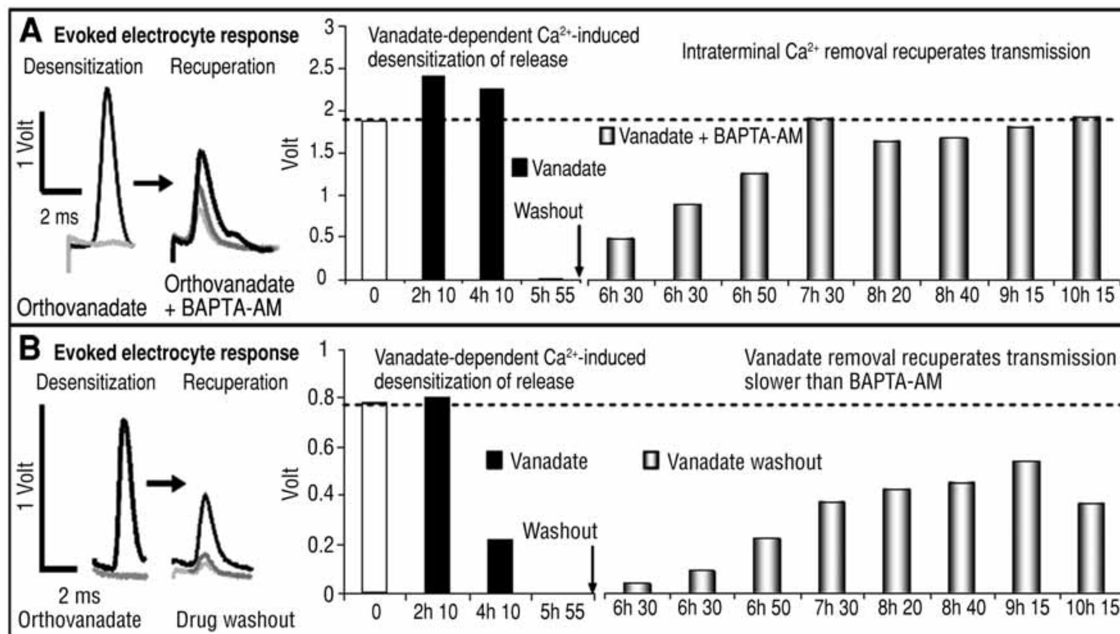

Fig. 2. *Torpedo* prisms were perfused continuously with marine solution containing 10 μ*M* Na^+-orthovandate (black bars). Evoked electrophysiological responses were recorded for several hours. Complete desensitization of release mechanism (after 5 h and 55 min) is shown in traces, followed by rapid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

in late Ca^{2+} homeostasis and extrusion (Dunant, this issue), working with Ca^{2+} ATPase in housekeeping tasks.

Acknowledgments

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