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PERSPECTIVE

Pharmacology Meets Vesicular Trafficking at a Central Nervous System Synapse: Pregabalin Effects on Synaptic Vesicle Cycling in Hippocampal Neurons

Ronald W. Holz

Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan

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ABSTRACT

The contribution by Micheva et al. (p. 467) in this issue of *Molecular Pharmacology* adds to our understanding of the action of pregabalin, a drug used for treatment of partial seizures and neuropathic pain. The authors examine the effects of pregabalin on presynaptic function of cultured hippocampal neurons using a powerful technique to follow the trafficking of synaptic vesicles in individual boutons. The study revealed that pregabalin reduces the readily releasable pool of synaptic vesicles in an *N*-methyl-D-aspartate receptor-dependent manner.

Pregabalin is a recently approved anticonvulsant and analgesic drug for use in the treatment of partial seizures and in the treatment of neuropathic pain due to diabetic and postherpes neuropathies. Its structure, function, and therapeutic uses are similar to those of gabapentin. Although both drugs were originally developed with the notion of specifically modulating GABAergic transmission, their biological and therapeutic effects were found to be independent of GABA receptors (both types A and B) and GABA uptake into neurons. Biochemical and electrophysiological studies found that the drugs caused small reductions in stimulated glutamate, norepinephrine, and peptide release from neurons, suggesting a basis for their therapeutic effects. Both drugs were unexpectedly bound with high affinity to the auxiliary Ca^{2+} channel subunit α_2-δ (type 1 or 2) (Gong et al., 2001; Piechan et al., 2004), raising the possibility that these agents act through modulation of voltage sensitive Ca^{2+} channels. Indeed, in some studies, but not in all, pregabalin and gabapentin cause partial inhibition of Ca^{2+} currents.

The contribution by (Micheva et al., 2006) in the current issue takes a fresh look at the action of pregabalin. The authors examine the effects of pregabalin on presynaptic function of cultured hippocampal neurons using a powerful technique to follow the trafficking of synaptic vesicles in individual boutons. The method uses amphipathic fluorescent dyes (FM dyes) that label endocytic vesicles in living neurons (Betz and Bewick, 1992; Rizzoli and Betz, 2005). When added to the extracellular medium, these membrane-impermeant probes equilibrate between the aqueous phase and the outer leaflet of the plasma membrane in which they become highly fluorescent (Fig. 1, C1). Upon endocytosis that follows exocytosis, the labeled membrane is internalized. When removed from the extracellular medium, the dye is retained by the endocytic vesicles in the bouton but is lost from the plasma membrane (Fig. 1, C2). Endocytic vesicles are transformed within approximately 1 min into synaptic vesicles containing the dye. Most importantly, these recycled synaptic vesicles lose the probe upon subsequent exocytosis when the vesicle membrane is exposed to the extracellular medium (Fig. 1, C3).

This technique has permitted the dynamics of the exocytic/endocytic cycle of synaptic vesicles to be investigated at individual boutons. In the cultured hippocampal neurons used in the study by Micheva et al. (2006), each bouton contains

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ABBREVIATIONS: FM 4-64, *N*-(3-triethylammoniumpropyl)-4-(6-(4-(diethylamino)phenyl)hexatrienyl)pyridinium dibromide; NMDA, *N*-methyl-D-aspartate.

approximately 200 synaptic vesicles (Fig. 1A). Half of these vesicles recycle upon stimulation at 37°C and are labeled upon incubation with FM dye (Fig. 1D) (Micheva and Smith, 2005). By labeling boutons by prior electrical stimulation in the presence of FM dye and then washing the cultures of extracellular dye, the subsequent exocytosis of synaptic vesicles can be followed by measuring the decrease in bouton fluorescence.

The present study by Micheva et al. (2006) made use of the unique strengths of this system to provide new insights into the action of pregabalin. By measuring fluorescence intensity changes in hundreds of individual boutons per experimental group with a time resolution of seconds, mechanistic insights were achieved despite the intrinsic variability of synaptic release. In fact, the remarkable statistical precision of the experiments in the hands of these investigators permitted the discovery of heretofore-unappreciated aspects of pregabalin effects.

Micheva et al. (2006) revealed a small inhibition of synaptic vesicle exocytosis at high stimulus frequency in both glutamatergic and GABAergic neurons at clinically relevant concentrations of pregabalin. The investigators were surprised to find that pregabalin targets a subpopulation of recycling vesicles, the readily releasable pool. This pool of vesicles is reduced by approximately 25%. Vesicles in the readily releasable pool are the first to be released upon electrical stimulation in the presence of Ca^{2+} and are specifically released in a Ca^{2+}-independent manner in response to hypertonic sucrose (Rosenmund and Stevens, 1996). Consistent with these results is the reduction in spontaneous synaptic vesicle exocytosis, which is known to be independent of Ca^{2+} and probably comes from the same readily releasable pool. These effects are unlikely to be caused by inhibition of voltage sensitive Ca^{2+} channels. They probably reflect a new action of pregabalin on the vesicular trafficking pathway in neurons, perhaps mediated by an unknown function of the auxiliary Ca^{2+} channel subunit α_2-δ. The effect of pregabalin to reduce the readily releasable pool is likely to have important functional implications. The reduction is predicted to decrease the probability of synaptic vesicle fusion even during a single action potential (Rosenmund and Stevens, 1996).

Another intriguing aspect of the study by Micheva et al. (2006) was the finding that an NMDA antagonist prevented the pregabalin-induced inhibition of transmission. Although NMDA receptors are primarily postsynaptic, there is also compelling evidence for presynaptic NMDA receptors (MacDermott et al., 1999). Thus, the NMDA receptor-mediated effect could originate either postsynaptically through a retrograde signaling pathway (Micheva et al., 2003) or presynaptically. It will be important in future studies to determine

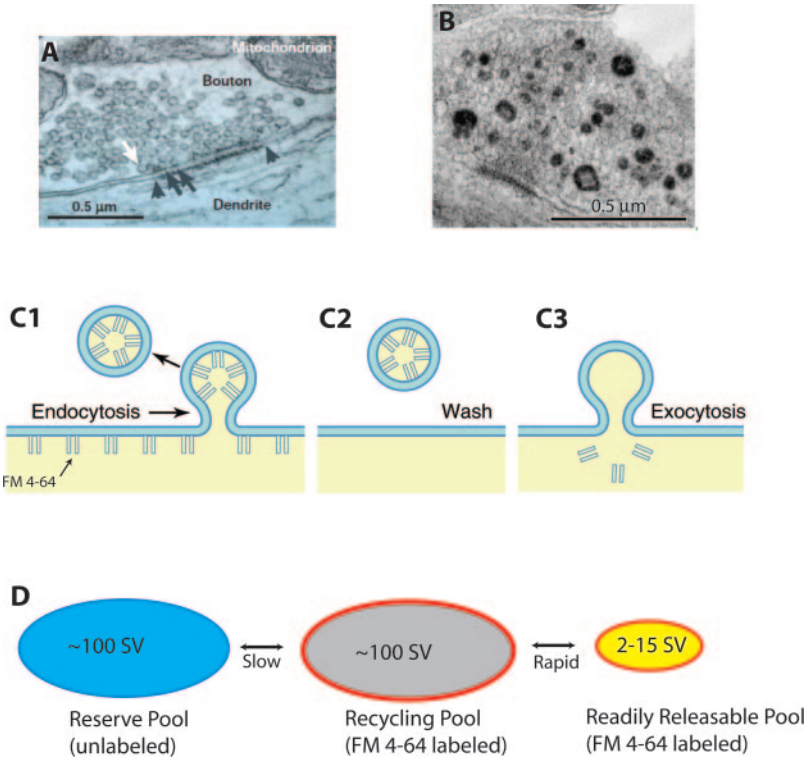

Fig. 1. Vesicular trafficking in hippocampal presynaptic boutons. A, electron micrograph of a bouton of a cultured hippocampal neuron. Arrowheads show edges of an active zone where exocytosis occurs. Black arrows indicate docked synaptic vesicles (SV), which are believed to represent the readily releasable pool. White arrow is a synaptic vesicle that is probably not docked. [Reproduced from Murthy VN, Schikorski T, Stevens CF, and Zhu Y (2001) Inactivity produces increases in neurotransmitter release and synapse size. *Neuron* **32:**673–682. Copyright © 2001 Elsevier. Used with permission.] B, horseradish peroxidase (HRP)-labeled structures in a bouton after electrical stimulation. HRP was present during electrical stimulation (10 Hz for 1 min) and for 1 min afterward (protocol is identical to that used for FM 4-64 labeling). HRP was detected by the dark appearance of reaction product from 3,3′-diaminobenzidine. Numerous structures were labeled including synaptic vesicles, clathrin-coated vesicles, and large vesicular structures. Quantitation of these micrographs as well as FM 4-64 labeling of living cells indicates that ~50% of the 200 SV in boutons recycle at 37°C. [Reproduced from Micheva KD and Smith SJ (2005) Strong effects of subphysiological temperature on the function and plasticity of mammalian presynaptic terminals. *J Neurosci* **25:**7481–7488. Copyright © 2005 Society for Neuroscience. Used with permission.] C, protocol for FM 4-64 staining of synaptic vesicles (adapted from Holz and Fisher, 2006). D, summary of different SV pools in cultured hippocampal boutons at 37°C. Data from Micheva and Smith (2005).

the mechanism by which NMDA receptor activation participates in pregabalin action.

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Address correspondence to: Ronald W. Holz, Department of Pharmacology, University of Michigan Medical School, 2301 MSRB III, Ann Arbor, MI 48109-0632. E-mail holz@umich.edu