Mind-altering miniature neurotransmitter release?

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It is well established that calcium is the trigger for fast action potential-evoked synaptic transmission (1). After elevation of intracellular calcium ($[Ca^{2+}]_i$) by action potential-mediated opening of voltage-dependent calcium channels (VDCCs), a low resting rate of neurotransmitter release of 0.01–0.03 vesicles per sec is elevated significantly to ≈20 per sec (2–4). Transmitter release occurring independently of action potential-mediated changes in $[Ca^{2+}]_i$ is termed "miniature release" and involves the stochastic release of individual vesicles (quanta). The quantal nature of miniature activity has been used to elucidate basic functional parameters of central nervous system (CNS) and neuromuscular synapses (5). Although miniature transmission can occur at basal $[Ca^{2+}]_i$ levels (≈80 nM), its frequency is greatly stimulated by even modest $[Ca^{2+}]_i$ elevation (<1 μM) (6). Miniature release has been proposed recently to have a role in maintaining the function of developing synapses during periods without action potential-evoked synaptic activity (refs. 7 and 8, but also see ref. 9) and is regulated in parallel to evoked release (10). In addition to being the trigger for fast chemical synaptic transmission, calcium is also required for coupling nerve-induced excitation to cardiac and smooth muscle contraction (11). As a treatment for hypertension and angina agents that interfere with calcium entry such as dihydropyridine (DHP), VDCC blockers are commonly used. Drugs with core 1,4-DHP structures potently block the L-type VDCC, which is required for muscle contraction. In the article by Hirasawa and Pittman (12) in this issue of PNAS, a paradoxical effect of the DHP nifedipine was found on miniature excitatory postsynaptic currents (mEPSCs) recorded from magnocellular neurons of the supraoptic nucleus of the hypothalamus. Specifically, nifedipine but not close chemical cousins such as nimodipine or nitrendipine potentially induces up to a 15-fold increase in the rate of miniature synaptic activity. Surprisingly, the effect of the drug has little to do with its action on the L-type VDCC, general calcium dynamics, or previously reported factors shown to affect mEPSC frequency such as nitric oxide (NO) and protein kinase cascades (Fig. 1). The authors imply that the drug may have a new potential site of action on the neurotransmitter release machinery itself or directly on the membrane fusion process. Although the exact mechanism is unclear, the results suggest that this commonly used therapeutic agent may have other mechanisms of action. Interestingly, the authors outline reports of CNS side effects apparently involving the DHP nifedipine (to a lesser extent than other DHPs) and imply that side effects could be attributed to modulation of miniature transmitter release.

Electrophysiological studies reveal multiple types (T, L, N, P, Q, and R) of VDCCs based on molecular, physiological, and pharmacological criteria (13). DHPs are well known classical organic ligands that bind specifically and with high affinity to L-type VDCCs in cardiac, skeletal, and smooth muscle and are used as antagonists. In these tissues blocking calcium entry reduces excessive contraction within the heart and decreases vascular tone. Therefore, DHPs such as nifedipine, nimodipine, and nitrendipine are important clinically for treatment of problems associated with the heart and circulatory system such as high blood pressure and angina. DHPs also block L-type VDCCs in CNS neurons and have been used to implicate the channel in stimulus transcription coupling (14). Regarding the mechanism by which nifedipine increases miniature synaptic activity, Hirasawa and Pittman (12) clearly show that it is completely independent of calcium-induced changes in basal release probability. The nifedipine-induced stimulatory effect on mEPSC frequency was not blocked by several strategies that interfere with calcium elevation including thapsigargin, a Ca^{2+}-ATPase inhibitor that depletes Ca^{2+} stores, chelation of $[Ca^{2+}]_i$ with 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate-acetoxymethyl ester (BAPTA-AM), or nonselective blockade of VDCCs with Cd^{2+}. To our knowledge there are a very few substances that can increase the frequency of miniature synaptic activity independently of changing $[Ca^{2+}]_i$. NO is a candidate, which through presumably cGMP-dependent signaling pathways can activate transmitter release (15). However, this mechanism was ruled out because the increase in mEPSC frequency was not blocked by an NO synthase inhibitor. Interestingly, NO generation induced by nifedipine has been reported in endothelial cells that do not express L-type VDCCs (16). The authors rule out direct non-NO synthase-dependent generation of NO by showing that NO donors only have minimal effects on mEPSC frequency. Another major mechanism for calcium-independent modulation of miniature transmitter release is through G proteins. However, G protein activation by ligands such as adenosine almost exclusively have a negative effect on mEPSC frequency (17). Hirasawa and Pittman (12) are quite thorough and exclude the possibility that nifedipine blocks adenosine action leading to a disinhibition of miniature release and an apparent increase in mEPSC frequency. Manipulations such as mild depolarization with potassium have been reported to elevate minifrequency (6). In this case VDCCs would be activated by direct potassium depolarization of the nerve terminal. However, such a depolarization by nifedipine seems unlikely, because Cd^{2+}-mediated antagonism of VDCCs does not alter the increase in mEPSC frequency by nifedipine. Depolarization of the terminal by blockade of small-conductance Ca^{2+}-activated K^+ channels through blockade of L-type VDCCs by nifedipine is also ruled out. The real novelty in the experiments of Hirasawa and Pittman (12) is that nifedipine provides a way of greatly increasing miniature synaptic activity frequency ap-

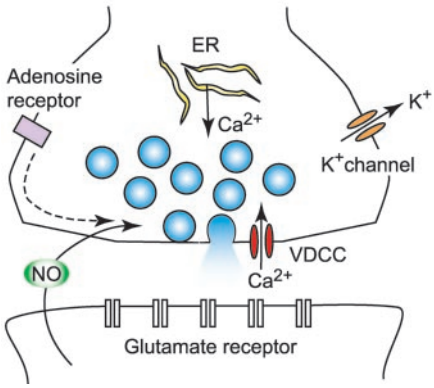

Fig. 1. Possible mechanisms for enhancement of mEPSC frequency: (*i*) increase in Ca^{2+} concentration in nerve terminal by activation of VDCC by membrane depolarization after blockade of K^+ channel and/or Ca^{2+} release from Ca^{2+} store; (*ii*) disinhibition of the adenosine system that normally inhibits mEPSC frequency; and (*iii*) NO, retrograde messenger. ER, endoplasmic reticulum.

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parently independently of altering $[Ca^{2+}]_i$. In pharmacology it is easy to imagine that drugs may have nonspecific effects that lead to inhibition of a process. For example, it would not be surprising that a pinch of dirt would inhibit miniature release, but an agent that produces a gain in function (independent of $[Ca^{2+}]_i$) is truly surprising. It is possible that through the use of nifedipine a novel target by which synaptic transmission can be manipulated independently of Ca^{2+} may be identified.

A major unresolved question is the site and mechanism of nifedipine action. The effect of nifedipine takes several minutes to be manifested and washes out slowly as well. Does this mean that the effect is within the plasma membrane or involves an intracellular cascade? The activation of protein kinase A or C pathways has been shown to facilitate transmitter release, and both are possible candidates (18, 19). However, the results of Hirasawa and Pittman (12) show that the nifedipine effect was independent of these pathways. Furthermore, because the effect of nifedipine was still observed after nicardipine treatment, it was thought to facilitate mEPSC frequency at a site different than the L-type VDCC. Hirasawa and Pittman also carefully ruled out potential contaminants in DHP preparations by checking material from different lot numbers as well as manufacturers. Perhaps the effect is similar to DMSO or alcohol in that it may increase miniature release by perturbing membrane structure (20, 21). However, this mechanism seems unlikely given that DMSO or alcohol act at much higher concentrations (in the range of 70–400 mM). Hypertonic sucrose is also an effective Ca^{2+}-independent experimental tool by which spontaneous or miniature release can be elevated. Hypertonic conditions are thought to induce a mechanical deformation of the active zone leading to release of a readily releasable pool of transmitter (22). Again the concentrations of nifedipine used (μM versus 100s mM) are inconsistent with promotion of miniature release through osmotic stress. In support of the mechanism being through an action of nifedipine on membrane lipids, Hirasawa and Pittman (12) reference an article that shows that different classes of DHP VDCC antagonists can have differential effects on membrane rigidity. In this study (23) nifedipine was compared with lacidipine and found to be considerably better at promoting membrane rigidity. Unfortunately, Hirasawa and Pittman (12) did not examine the effects of DHPs with differential effects on membrane rigidity (nifedipine versus lacidipine) on miniature release. α-Latrotoxin, a black widow spider neurotoxin, is well studied and known to bind to the nerve terminal leading to stimulation of amino acid transmitter release machinery independently of intra- and extracellular Ca^{2+} (24). It is conceivable that nifedipine is able to interact with proteins associated with the release apparatus, and thus it may be useful for the study of release mechanisms.

Hirasawa and Pittman (12) argue that potential CNS side effects of nifedipine could be linked to its action on miniature synaptic activity. Evidence for this proposal is derived from a web site documenting adverse reactions to calcium channel blockers (www.drugdigest.org/DD/comparison/NewComparison/0,10621,26-17,00/html). Clearly the data are suggestive, although more conclusive studies are required to examine a causal link to nifedipine use and its CNS side effects. It is also noteworthy that patients receiving nifedipine are in general elderly and may already be subject to confusion, dizziness, or other perceived CNS side effects. In addition, the CNS side effects could be secondary to the efficacy of nifedipine at lowering blood pressure. If nifedipine-specific CNS drug side effects can be documented better, the question of whether this has anything to do with the action of nifedipine on miniature transmission is still a difficult one to address. Typically miniature release only occurs at a frequency of a few hertz within a neuron and is of insufficient amplitude to evoke a postsynaptic action potential in a principal neuron by itself. However, the firing of electrically compact interneurons (that are close to threshold) can be influenced by individual quantal release events (25). These findings raise the question of what the functional significance of miniature synaptic activity is in large principal neurons. Interestingly, miniature release is able to activate synapse-specific second messengers such as calcium calmodulin-dependent protein kinase II (26, 27) and can be associated with a cell culture form of long-term potentiation (28, 29). Although not examined explicitly by the authors, it is also possible that nifedipine potentiates evoked synaptic activity, which could be a major contributor to its CNS side effects. In the end, this study may provide another tool to better understand the role of miniature synaptic activity in brain function. Perhaps the use of novel pharmacological tools such as nifedipine coupled with specific synaptic protein mutants (30) will be used in the future as paradigms to determine whether miniature synaptic activity is merely a useless byproduct of the mechanism of chemical transmission or whether it represents a secondary means of signaling with distinct functional consequences.

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