Electrophysiological studies have defined several pharmacologically distinct high-voltage-activated (HVA) Ca\(^{2+}\) channels on neuronal cell bodies (1). Among these are L-, N-, and P-type channels which are the best known (1), but other classes of channels have been revealed by molecular cloning and electrophysiology (2-7). How diverse Ca\(^{2+}\) channels contribute to synaptic transmission in the central nervous system is not entirely clear (7). We studied the synapse between hippocampal CA3 and CA1 neurons (8), a focus of interest in examination of glutamatergic transmission and synaptic plasticity (9). At this synapse, inhibition of L-type channels by nifedipine has little effect (10), and much of the transmission remains after blockade of N-type channels by ω-Conotoxin GVIA (ω-CTX-GVIA) (10, 11). We found that the Ca\(^{2+}\) channels that mediate the remaining transmission are pharmacologically distinct from classical L- and P-type Ca\(^{2+}\) channels. Instead, their pharmacological profile resembled that of α1A Ca\(^{2+}\) channel subunits expressed in Xenopus oocytes (4) and the Q-type Ca\(^{2+}\) channel current in cerebellar granule neurons (6). Stimulation of neurotransmitter receptors can greatly attenuate synaptic transmission mediated by α1A channels. Reductions in the contribution of either Q- or N-type channels greatly increased the degree to which closely spaced stimuli facilitated synaptic transmission. These results suggest that cooperation may occur among multiple Ca\(^{2+}\) channel types in the control of transmitter release and may be advantageous for precise regulation of the strength- or frequency-dependence of synaptic function.

Selective blockade of N-type Ca\(^{2+}\) channels with 1 μM ω-Ctx-GVIA (12) caused a rapid but incomplete depression of synaptic transmission (Fig. 1A), which was not reversed by extensive washing. This block was maximal, inasmuch as a higher concentration of toxin (3 μM) produced no additional inhibition (Fig. 1A). The average degree of inhibition was 46 ± 1% (n = 30) (Fig. 1B). We tested agents that influence other classes of Ca\(^{2+}\) channels to determine which might be responsible for the remaining transmission. Synaptic transmission was unaffected by FPL 64176, a powerful agonist of L-type channels (n = 4) (13), or nifedipine, a specific blocker of L-type channels (n = 6) (Fig. 1C). Likewise, application of 30 nM ω-Agatoxin IVA (ω-Aga-IVA), which potently blocks P-type Ca\(^{2+}\) channels (14), had no effect on transmission (Fig. 1D) (15). This was true regardless of whether ω-Aga-IVA was applied before (n = 14) or after (n = 2) ω-CTX-GVIA. The ω-CTX-GVIA- and ω-Aga-IVA-resistant transmission was completely and reversibly eliminated by removal of external Ca\(^{2+}\) ions (Fig. 1D). These experiments demonstrate that substantial excitatory synaptic transmission can be supported by a Ca\(^{2+}\) channel that is not of the N-, L-, or P-type.

The Q- and R-type Ca\(^{2+}\) channels in cerebellar granule neurons are resistant to blockade by ω-CTX-GVIA, nimodipine, and ω-Aga-IVA at concentrations sufficient to eliminate N-, L-, and P-type channels, respectively (5, 6). The Q- and R-type Ca\(^{2+}\) channels appear to be generated by α1A and α1E subunits (3-5). The Q-type channels are completely blocked by 1.5 μM ω-CTX-MVIIC and are largely suppressed by ω-Aga-IVA at 1 μM (4, 6), a concentration 100 to 1000 times that needed to block P-type channels (14). In contrast, R-type channels are little affected by either of these treatments (5). These characteristics enabled us to determine the contribution of Q- or R-type channels to hippocampal synaptic transmission. Application of 5 μM ω-CTX-MVIIC completely abolished the transmission that was not mediated by N-, L-, or P-type Ca\(^{2+}\) channels (n = 4) (Fig. 2A). Neuronal excitability was unaffected by toxin, as indicated by increases in the amplitude of excitatory postsynaptic potentials and unconditional firing rates of CA1 pyramidal neurons (26).
cated by extracellular recordings of the presynaptic volley or postsynaptic action potentials elicited by antidromic stimulation. Synaptic transmission could not be restored even with trains of presynaptic impulses or increased extracellular Ca\(^{2+}\) concentration (16). The blockade by \(\omega\)-CTX-MVIIC increased in speed and completeness as its concentration was raised from 150 nM to 5 \(\mu\)M (\(n = 2 \text{ to } 10\)) (Fig. 2B). The dose dependence resembled that found for \(\omega\)-CTX-MVIIC blockade of Q-type current in cultured cerebellar granule cells (6) and \(\alpha_{1A}\) currents expressed in Xenopus oocytes (4). Calcium channel currents with the properties of currents carried by Q-type channels have been described for CA3 cell bodies (16, 17).

Both N- and Q-type Ca\(^{2+}\) channels are blocked by \(\omega\)-CTX-MVIIC (4, 6, 16–18). If N- and Q-type channels account complete-

**Fig. 1.** Effects of Ca\(^{2+}\) channel blockers on excitatory synaptic transmission. (A) Synaptic strength (monitored as maximal slope of the field EPSP) recorded in hippocampal area CA1 during 0.033-Hz stimulation of the Schaffer collateral and commissural pathways. Typical response to application of \(\omega\)-CTX-GVIA. (Inset) Averages of 10 consecutive responses acquired at the times indicated. (B) Averaged response to \(\omega\)-CTX-GVIA (\(n = 30\)). Field EPSP slope values were normalized to their mean value during the 5 min preceding application of toxin. Symbols represent mean ± SEM. (C) Test for involvement of L-type channels, with the L-type–specific agonist FPL 64176 and the antagonist nimodipine. (D) Effect of \(\omega\)-Aga-IVA, an inhibitor of P-type Ca\(^{2+}\) channels, applied before and after exposure to \(\omega\)-CTX-GVIA. The effect of removing external Ca\(^{2+}\) ions on \(\omega\)-CTX-GVIA–insensitive transmission is also shown.

**Fig. 2.** Block of \(\omega\)-CTX-GVIA–insensitive synaptic transmission by an inhibitor of Q-type Ca\(^{2+}\) channels, \(\omega\)-CTX-MVIIC. (A) Effect of \(\omega\)-CTX-MVIIC after complete blockade of N-type Ca\(^{2+}\) channels with \(\omega\)-CTX-GVIA. (B) Ensemble averages of responses to \(\omega\)-CTX-MVIIC after application of \(\omega\)-CTX-GVIA. ○, 150 nM (\(n = 2\)); ●, 500 nM (\(n = 2\)); ▲, 1.5 \(\mu\)M (\(n = 2\)); □, 5 \(\mu\)M (\(n = 10\)). (C) Effect of application of \(\omega\)-CTX-MVIIC before \(\omega\)-CTX-GVIA. (D) Average effect of \(\omega\)-Aga-IVA on control transmission. ○, 200 nM (\(n = 2\)); ●, 300 nM (\(n = 2\)); ▲, 1.0 \(\mu\)M (\(n = 5\)).
ly for the transmitter release, then transmission should be abolished by application of \( \omega \text{-Ctx-MVIIC} \) alone. Indeed, 5 \( \mu \text{M} \) \( \omega \text{-Ctx-MVIIC} \) completely blocked synaptic transmission (\( n = 6 \)) (Fig. 2C). The inhibitory effects of \( \omega \text{-Ctx-MVIIC} \) on control transmission were dose-dependent (150 nM \( \omega \text{-Ctx-MVIIC} \) produced 76 ± 2% block, \( n = 6 \)) (16). Removal of the toxin was followed by partial restoration of transmission, presumably reflecting recovery of N-type channels which recover more quickly than Q-type channels from inhibition by \( \omega \text{-Ctx-MVIIC} \) (18). Subsequent application of \( \omega \text{-Ctx-GVIA} \) abolished the transmission that recovered during washout of \( \omega \text{-Ctx-MVIIC} \) (\( n = 10 \)) (Fig. 2C), confirming the contribution of N-type channels to recovery. These data support the hypothesis that Q- and N-type channels account entirely for synaptic transmission.

At doses of up to \( \sim 1 \mu \text{M} \), Aga-IVA blocks Q-type (and P-type) channels but spares N-type channels (19). Thus, Aga-IVA provides a means of isolating transmission supported by N-type channels alone. Synaptic transmission was inhibited with Aga-IVA (1 \( \mu \text{M} \)) by 85 ± 4% (\( n = 5 \)) (Fig. 2D). Lower doses of the toxin (200 or 300 nM) caused partial blockade of transmission that did not attain steady state, even after a 90-min application (\( n = 4 \)). The requirement for relatively high toxin concentrations and the slow time course of block stand in contrast to the effects of Aga-IVA in systems where P-type channels have been implicated in transmitter release (20). Instead, the kinetics of Aga-IVA inhibition found here are consistent with properties of Q-type channels (4, 6).

We tested whether transmitter release triggered by Q-type \( \text{Ca}^{2+} \) channels is responsive to neuromodulators known to affect other HVA \( \text{Ca}^{2+} \) channels (21) by monitoring the field excitatory postsynaptic potential (EPSP) slope with \( \omega \text{-Ctx-GVIA} \) present to block N-type \( \text{Ca}^{2+} \) channels. Stimulation of metabotropic glutamate (mGlu), \( \gamma \)-aminobutyric acid type B (GABA\(_B\)), adenosine, or acetylcholine (ACh) receptors depressed synaptic transmission (Fig. 3A). Pooled results from many slices demonstrated the consistency of these effects (Fig. 3, C through F). Activation of protein kinase C (PKC) produces stimulatory effects on N- and L-type \( \text{Ca}^{2+} \) channels (22) and hippocampal synaptic transmission (11, 23). We examined the effect of phorbol 12,13-dibutyrate (PDBu), an activator of PKC, on transmission supported only by Q-type channels. After application of \( \omega \text{-Ctx-GVIA} \), addition of PDBu first produced a small, transient depression (14 ± 2%) and then a large, sustained potentiation of the synaptic response (222 ± 31%, \( n = 4 \)) (Fig. 3B). Neither of these changes was seen with 4a-PDBu, a congener of PDBu that does not stimulate PKC (\( n = 4 \)) (16). The PDBu-potentiated transmission was unaffected by further exposure to \( \omega \text{-Ctx-GVIA} \) (\( n = 4 \)) (16) but was completely abolished by \( \omega \text{-Ctx-MVIIC} \) (Fig. 3B).

Prior application of PDBu reduced or abolished modulation of transmission by stimulation of neurotransmitter receptors (Fig. 3, C through F). These effects were not observed when 4a-PDBu was applied (\( n = 2 \)). An interaction between PKC and neurotransmitter effects has been found for transmission.

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**Fig. 3.** Modulation of synaptic transmission supported by Q-type \( \text{Ca}^{2+} \) channels by agonists of common neurotransmitter receptors. (A) Effects of stimulating mGlu (1S,3R-ACPD), GABA\(_B\) (\( \text{(-)} \)-baclofen but not \( \text{(+)} \)-baclofen; \( n = 4 \)), adenosine [2-chloroadenosine (2-CA)], and ACh [carbachol (Carb)] receptors on synaptic transmission after blockade of N-type \( \text{Ca}^{2+} \) channels with \( \omega \text{-Ctx-GVIA} \). Also note the lack of effect of a second application of \( \omega \text{-Ctx-GVIA} \) 4 hours after the first application. (B) Ensemble average of experiments in which persistent enhancement of synaptic strength was induced, after application of \( \omega \text{-Ctx-GVIA} \), by continuous application of PDBu (\( n = 4 \)). The synaptic responses were depressed by 14 ± 2% at 10 to 15 min after PDBu and enhanced by 222 ± 31% at 90 min. Time-aligned and ensemble-averaged data to the right. (C) Average response to 10-min application of 1S,3R-ACPD in the absence (\( \mathcal{O} \), 56 ± 4% block, \( n = 10 \)) or presence (\( \mathcal{P} \), 5 ± 1% block, \( n = 4 \)) of PDBu. (D) Average response to 5-min application of \( \text{(-)} \)-baclofen in the absence (\( \mathcal{O} \), 75 ± 3% block, \( n = 12 \)) or presence (\( \mathcal{P} \), 5 ± 2% block, \( n = 4 \)) of PDBu. (E) Average response to 5-min application of 2-CA in the absence (\( \mathcal{O} \), 83 ± 4% block, \( n = 6 \)) or presence (\( \mathcal{P} \), 28 ± 5% block, \( n = 4 \)) of PDBu. (F) Average response to 10-min application of carbachol in the absence (\( \mathcal{O} \), 54 ± 4% block, \( n = 6 \)) or presence (\( \mathcal{P} \), 14 ± 4% block, \( n = 4 \)) of PDBu. In (C) through (F), the error bars are smaller than the symbols.
at other synapses (22) and has been attributed to modulatory effects on Ca\(^{2+}\) channels, primarily of the N-type (22, 24). A similar convergence of signaling pathways may also hold for Q-type channels (25).

Hippocampal synapses display several forms of plasticity, including paired-pulse facilitation (PPF) and long-term potentiation (LTP). We assessed the ability of synaptic transmission mediated by individual Ca\(^{2+}\) channel types to undergo such changes in synaptic strength. Synaptic responses to a pair of stimuli were recorded before and after isolation of transmission mediated by Q- or N-type channels (Fig. 4A). The PPF was taken as the percent increase in the second response relative to the first. It was increased from 40 ± 4% to 61 ± 5% by application of 1 μM ω-Ctx-GVIA, and from 39 ± 7% to 77 ± 8% by exposure to 1 μM ω-Aga-IVA (Fig. 4B) (26). Control versus ω-Ctx-GVIA, paired t(14) = 4.013, P < 0.01; control versus ω-Aga-IVA, paired t(5) = 17.5, P < 0.0001. Thus, PPF is more pronounced for synaptic transmission mediated solely by Q- or N-type channels than for transmission supported by both channel types together.

After application of ω-Ctx-GVIA to isolate synaptic transmission mediated solely by Q-type Ca\(^{2+}\) channels, tetanic stimulation in one pathway induced a consistent and sustained synaptic enhancement (38 ± 1% at 30 min post-tetanus, n = 5), whereas transmission remained stable in an untetanized, independent pathway (Fig. 4C). The synaptic transmission in both control and potentiated pathways was completely eliminated by ω-Ctx-MVIIC (n = 3) (16). This suggests that the glutamate release triggered by Q-type channels is sufficient to allow both induction and expression of LTP. Because the expression of LTP is at least partly presynaptic (27), we tested whether the presynaptic enhancement might arise from an increase in the efficacy of either N- or Q-type Ca\(^{2+}\) channels. After induction of LTP in the absence of toxin, the contribution of N-type channels was determined by application of ω-Ctx-GVIA. The resulting reduction in synaptic strength was indistinguishable in control and potentiated pathways (49 ± 2% in both cases, n = 4) (Fig. 4D). Thus, the relative contribution of N- and Q-type Ca\(^{2+}\) channels remained unchanged during LTP. A uniform enhancement of both channel types, however, was not excluded. Because the induction, maintenance, and expression of LTP were not significantly affected by the presence of ω-Ctx-GVIA [control versus ω-Ctx-GVIA, unpaired t(4) = 1.576, P = 0.17], our results rule out any essential role of N-type channels in LTP. Fluorometric measurements in Schaffer collateral axons have shown no change in the transient increase in presynaptic Ca\(^{2+}\) concentration after induction of LTP (28). These findings argue against any change in presynaptic Ca\(^{2+}\) delivery during LTP (29), while leaving open the possibility of a change in Ca\(^{2+}\) sensitivity or the magnitude of the Ca\(^{2+}\)-dependent response.

The pharmacological profile for synaptic transmission at hippocampal CA3-CA1 synapses reveals that one of the Ca\(^{2+}\) channels that mediates transmitter release has properties like the α1A Ca\(^{2+}\) channel subunit expressed in Xenopus oocytes (4), and Q-type channels in cerebellar granule neurons (6). This delineates a physiological role for the α1A subunit as a mediator of synaptic transmission. Although the α1A Q-type Ca\(^{2+}\) channel appears dominant under our experimental conditions, N-type Ca\(^{2+}\) channels also participate. There was no indication of any involvement of L- or P-type channels. These conclusions about electrically evoked glutamatergic transmission in hippocampal slices differ from those drawn from characterization of glutamate release from rat cortical synaptosomes stimulated with increased concentrations of external K\(^{+}\) (30). The synaptic release was not affected by ω-Ctx-GVIA but was reduced by 30 to 300 nM ω-Aga-IVA. Increased external K\(^{+}\) concentrations produce a depolarization that is longer but weaker than that of an action potential and may thus recruit a different set of Ca\(^{2+}\) channels. Also, transmitter release at different synapses within the brain may rely on different subsets of Ca\(^{2+}\) channels (31).

These results appear to be inconsistent with N- and Q-type channels acting independently at two nonoverlapping populations of release sites. If this were the case, the effects of selective and complete blockade of N- and Q-type channels would be additive. Additionally, an excess of PPF at one kind of release site would be balanced by below average PPF at the other. On the contrary, selective blockade of Q- or N-type channels produced reductions in synaptic strength whose sum exceeded 100%, and PPF of synaptic transmission mediated by either channel type in isolation was greater than that of control transmission. The observations can be understood...
stood if Q- and N-type Ca$^{2+}$ channels are localized in proximity and cooperate in the delivery of Ca$^{2+}$ to individual release sites (31). Because transmitter release depends on the local Ca$^{2+}$ concentration raised to a power $n > 2$ (32), elimination of Ca$^{2+}$ entry through either type of channel would produce a disproportionately large reduction in synaptic strength (33). Furthermore, when transmission is diminished by isolation of a single class of channels, PPF would be enhanced by the reduction in Ca$^{2+}$ entry, much as it is with lowered extracellular Ca$^{2+}$ concentration (29, 34).

Synaptic transmission mediated solely by Q-type channels was susceptible to various forms of modulation. Activators of mGlut, GABA$_A$ adenosine, or ACh receptors strongly inhibited such transmission, whereas PKC enhanced it and opposed the receptor-mediated inhibition. All of these characteristics have been attributed to N-type channels (21, 22, 24). Our results indicate that this cannot be the sole explanation and suggest that modulation of Q-type Ca$^{2+}$ channels may also be important for regulation of synaptic strength (25). This is consistent with evidence for current modulation through Ca$^{2+}$ channels that are not of the L-, N-, or P-type in cerebellar granule cells (35), hippocampal pyramidal cells, and spinal interneurons (14, 36). The finding that neurotransmitter release depends heavily on the cooperative actions of more than one class of Ca$^{2+}$ channel increases our knowledge of possible avenues for physiological regulation of synaptic transmission by neuroactive substances.


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<td>8. Transverse hippocampal slices (400 µm) were obtained by standard methods [B. E. Alger et al., in Brain Slice Methods, R. Dingledine, Ed. (Thorn, New York, 1984), pp. 361–438; from 3- to 4-week-old Sprague-Dawley rats that were housed and killed in accordance with the guidelines set forth by the Stanford University Administrative Panel on Laboratory Animal Care. Rats were anesthetized with halothane and hippocampi were quickly dissected and sliced in cold Krebs buffer containing 130 mM NaCl, 2.5 mM KCl, 1.3 mM MgCl$_2$, 2.0 mM CaCl$_2$, 1.0 mM Na$_2$PO$_4$, 26.2 mM NaHCO$_3$, and 11.0 mM glucose and gassed with 95% O$_2$ and 5% CO$_2$.</td>
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Roles of N-type and Q-type Ca2+ channels in supporting hippocampal synaptic transmission
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