reports are appearing that FGF-2 may cause up-regulation of its own message and protein, at least in cells in culture [J. C. Fox and J. L. Swain, *In Vitro Cell. Dev. Biol.* **29A**, 228 (1993); B. Flott-Rahmel *et al.*, *Neuroreport* **3**, 1077 (1992)]. This must be explored further in the limb bud.

11. Fertile White Leghorn chicken eggs were incubated at 38°C for 3 days [stage 20, V. Hamburger and H. L. Hamilton, *J. Morphol.* **88**, 49 (1952)]. After staining with Nile blue, the apical ridge was removed with a tungsten needle. Controls were either reincubated without further treatment or received an Affi-gel blue bead soaked in phosphate-buffered saline (PBS). FGF-2–loaded beads were grafted immediately following ridge removal. Affi-gel blue beads (Bio-Rad) 200 μm in diameter were incubated in either PBS or 0.56 nmol human recombinant FGF-2 for 1 hour at 37°C.
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13. Human recombinant FGF-2 was iodinated as described [B. B. Olwin and S. D. Hauschka, *J. Cell Biol.* **110**, 503 (1990)] except that ^{125}I-labeled FGF-2 was not purified from free ^{125}I, and no carrier protein was used. Affi-gel blue beads were incubated in iodinated FGF-2 (0.57 μg, 6.1 × 10^5 cpm/μl) for 1 hour at 37°C, agitating every 10 min. Beads were rinsed and counted individually in a Wallac Clinigamma counter for 1 min. Selected beads were implanted into the right wing bud of stage 20 embryos, which were then reincubated for 24, 48, 72, or 96 hours, when the bead was removed from the wing and the amount of radioactivity in the bead determined.
14. Forty 200-μm Affi-gel blue beads incubated in iodinated FGF-2 (32 pmol in 100 μl) adsorbed 18% of the iodinated FGF-2, yielding 0.14 pmol/bead (2 × 10^5 cpm per bead). Incubation of 40 beads in 0.23 pmol unlabeled FGF-2 containing iodinated FGF-2 yielded a nearly identical incorporation of 19%. It is unlikely the beads are saturated and thus they will incorporate similar amounts of FGF-2 at low concentrations (30 pmol) used to examine release in vivo or higher concentrations (0.6 nmol) used to replace apical ridge function. Incubation of beads in 1 ml of 0.56 mmol unlabeled FGF-2 then yields 2.4 pmol loaded per bead [compare with A. Hayek *et al.*, *Biochem. Biophys. Res. Comm.* **147**, 876 (1987)].
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Roles of N-Type and Q-Type Ca^{2+} Channels in Supporting Hippocampal Synaptic Transmission

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Several types of calcium channels found in the central nervous system are possible participants in triggering neurotransmitter release. Synaptic transmission between hippocampal CA3 and CA1 neurons was mediated by N-type calcium channels, together with calcium channels whose pharmacology differs from that of L- and P-type channels but resembles that of the Q-type channel encoded by the α_{1A} subunit gene. Blockade of either population of channels strongly increased enhancement of synaptic transmission with repetitive stimuli. Even after complete blockade of N-type channels, transmission was strongly modulated by stimulation of neurotransmitter receptors or protein kinase C. These findings suggest a role for α_{1A} subunits in synaptic transmission and support the idea that neurotransmitter release may depend on multiple types of calcium channels under physiological conditions.

Electrophysiological studies have defined several pharmacologically distinct high voltage–activated (HVA) Ca^{2+} channels on neuronal cell bodies (*1*). Among these the L-, N-, and P-type channels 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 α_{1A} 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 α_{1A} 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 nimodipine, 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 α_{1A} and α_{1E} 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 = 14) (Fig. 2A). Neuronal excitability was unaffected by toxin, as indi-

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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 ω-CTx-MVIIC increased in speed and completeness as its concentration was raised from 150 nM to 5 μM (n = 2 to 10) (Fig. 2B). The dose dependence resembled that found for ω-CTx-MVIIC blockade of Q-type current in cultured cerebellar granule cells (6) and α_{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 ω-CTx-MVIIC (*4*, *6*, *16*–*18*). If N- and Q-type channels account complete-

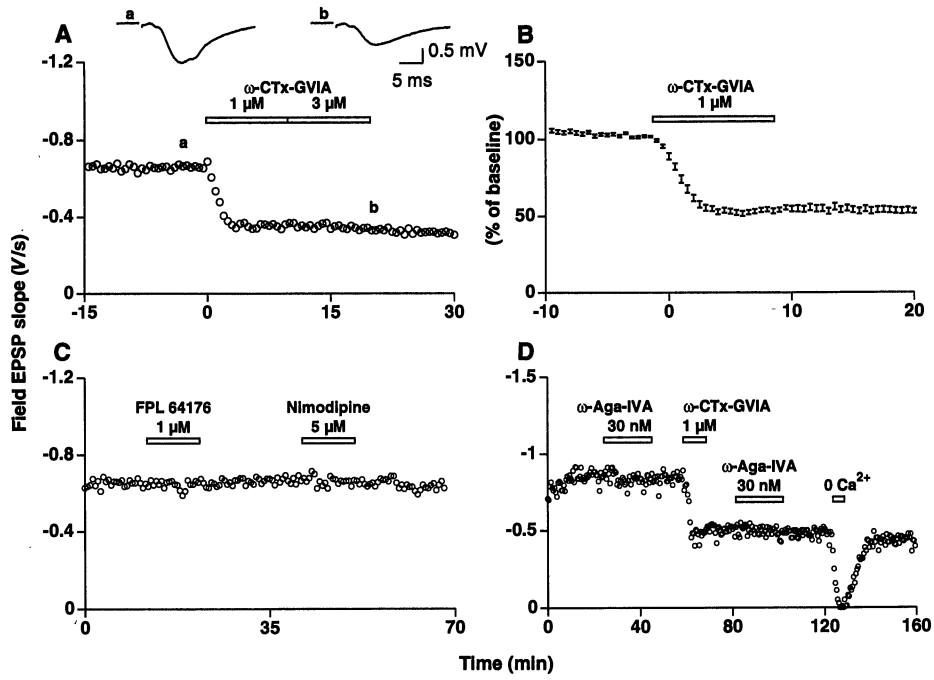

Fig. 1. Effects of Ca^{2+} channel blockers on excitatory synaptic transmission. (**A**) Synaptic strength (monitored as maximal slope of the field EPSP) recorded in hippocampal area CA1 during 0.033-Hz stimulation of the Schaffer collateral and commisural pathways. Typical response to application of ω-CTx-GVIA. (**Inset**) Averages of 10 consecutive responses acquired at the times indicated. (**B**) Averaged response to ω-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 ω-Aga-IVA, an inhibitor of P-type Ca^{2+} channels, applied before and after exposure to ω-CTx-GVIA. The effect of removing external Ca^{2+} ions on ω-CTx-GVIA–insensitive transmission is also shown.

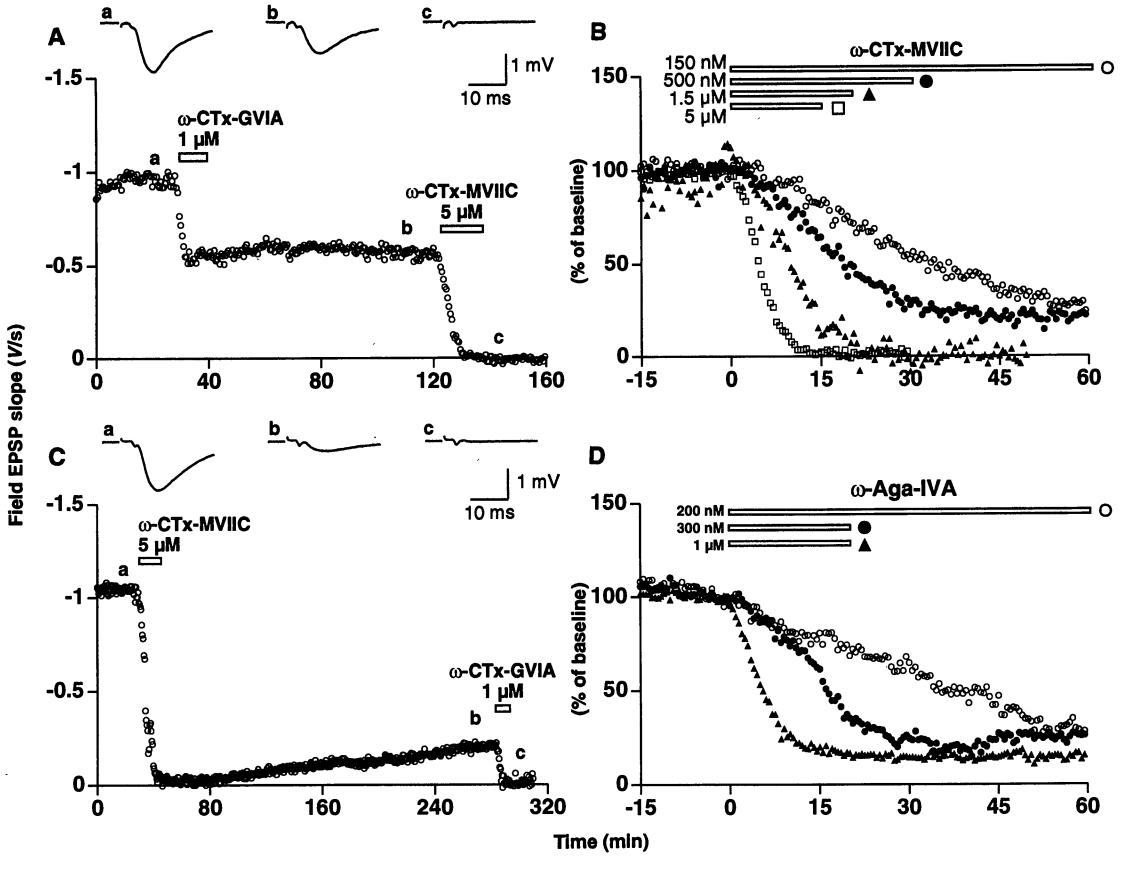

Fig. 2. Block of ω-CTx-GVIA–insensitive synaptic transmission by an inhibitor of Q-type Ca^{2+} channels, ω-CTx-MVIIC. (**A**) Effect of ω-CTx-MVIIC after complete blockade of N-type Ca^{2+} channels with ω-CTx-GVIA. (**B**) Ensemble averages of responses to ω-CTx-MVIIC after application of ω-CTx-GVIA. ○, 150 nM (n = 2); ●, 500 nM (n = 2); ▲, 1.5 μM (n = 2); □, 5 μM (n = 10). (**C**) Effect of application of ω-CTx-MVIIC before ω-CTx-GVIA. (**D**) Average effect of ω-Aga-IVA on control transmission. ○, 200 nM (n = 2); ●, 300 nM (n = 2); ▲, 1.0 μM (n = 5).

ly for the transmitter release, then transmission should be abolished by application of ω-CTx-MVIIC alone. Indeed, 5 μM ω-CTx-MVIIC completely blocked synaptic transmission (n = 6) (Fig. 2C). The inhibitory effects of ω-CTx-MVIIC on control transmission were dose-dependent (150 nM ω-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 ω-CTx-MVIIC (*18*). Subsequent application of ω-CTx-GVIA abolished the transmission that recovered during washout of ω-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.

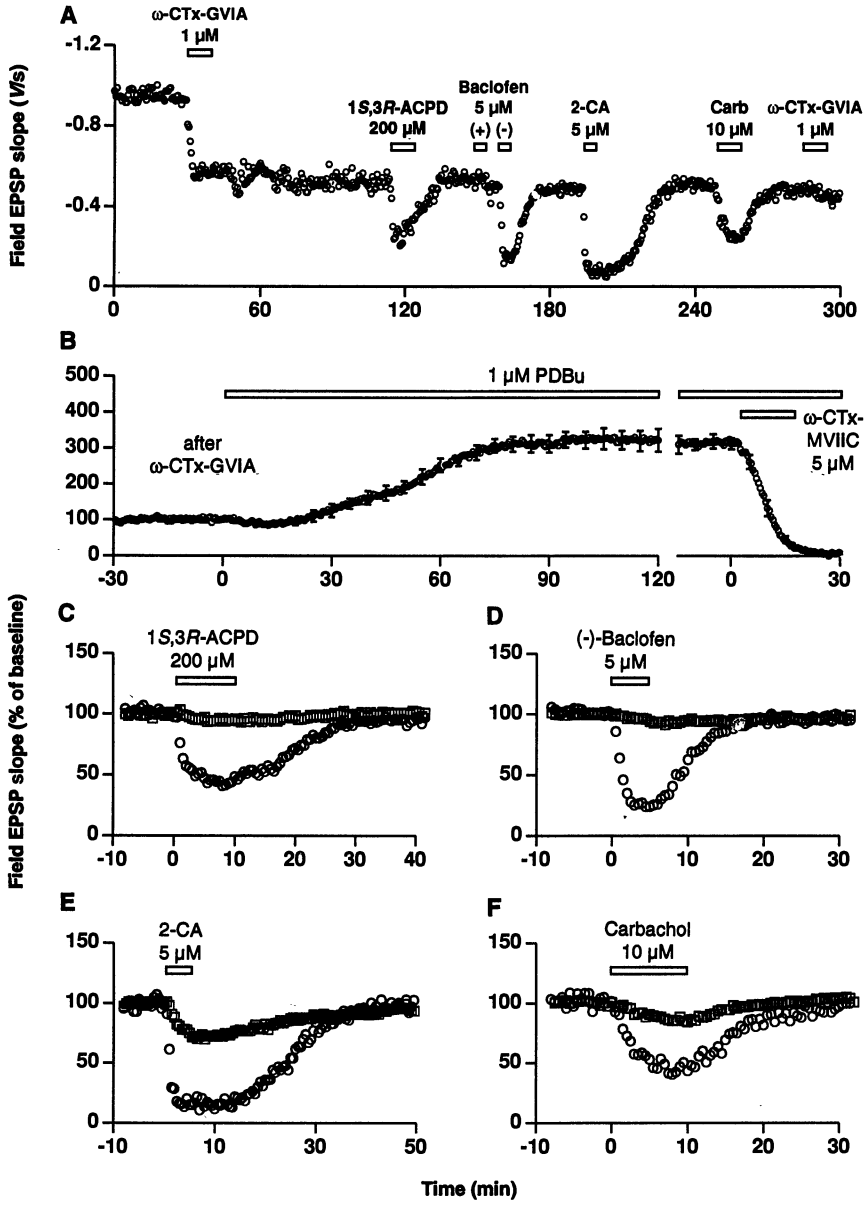

Fig. 3. Modulation of synaptic transmission supported by Q-type Ca^{2+} channels by agonists of common neurotransmitter receptors. (**A**) Effects of stimulating mGlu (1*S*,3*R*-ACPD), $GABA_B$[(−)-baclofen but not (+)-baclofen; n = 4], adenosine [2-chloroadenosine (2-CA)], and ACh [carbachol (Carb)] receptors on synaptic transmission after blockade of N-type Ca^{2+} channels with ω-CTx-GVIA. Also note the lack of effect of a second application of ω-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 ω-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 1*S*,3*R*-ACPD in the absence (○, 56 ± 4% block, n = 10) or presence (□, 5 ± 1% block, n = 4) of PDBu. (**D**) Average response to 5-min application of (−)-baclofen in the absence (○, 75 ± 3% block, n = 12) or presence (□, 5 ± 2% block, n = 4) of PDBu. (**E**) Average response to 5-min application of 2-CA in the absence (○, 83 ± 4% block, n = 6) or presence (□, 28 ± 5% block, n = 4) of PDBu. (**F**) Average response to 10-min application of carbachol in the absence (○, 54 ± 4% block, n = 6) or presence (□, 14 ± 4% block, n = 4) of PDBu. In (C) through (F), the error bars are smaller than the symbols.

At doses of up to ~1 μM, ω-Aga-IVA blocks Q-type (and P-type) channels but spares N-type channels (*19*). Thus, ω-Aga-IVA provides a means of isolating transmission supported by N-type channels alone. Synaptic transmission was inhibited with ω-Aga-IVA (1 μM) 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 ω-Aga-IVA in systems where P-type channels have been implicated in transmitter release (*20*). Instead, the kinetics of ω-Aga-IVA inhibition found here are consistent with properties of Q-type channels (*4*, *6*).

We tested whether transmitter release triggered by Q-type Ca^{2+} channels is responsive to neuromodulators known to affect other HVA Ca^{2+} channels (*21*) by monitoring the field excitatory postsynaptic potential (EPSP) slope with ω-CTx-GVIA present to block N-type Ca^{2+} channels. Stimulation of metabotropic glutamate (mGlu), γ-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 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 ω-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 4α-PDBu, a congener of PDBu that does not stimulate PKC (n = 4) (*16*). The PDBu-potentiated transmission was unaffected by further exposure to ω-CTx-GVIA (n = 4) (*16*) but was completely abolished by ω-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 4α-PDBu was applied (n = 2). An interaction between PKC and neurotransmitter effects has been found for transmission

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 α_{1A} 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 α_{1A} subunit as a mediator of synaptic transmission. Although the α_{1A} 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 synaptosomal release was not affected by ω-CTx-GVIA but was reduced by 30 to 300 nM ω-Aga-IVA. Increased external K^+ concentration produces 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 under-

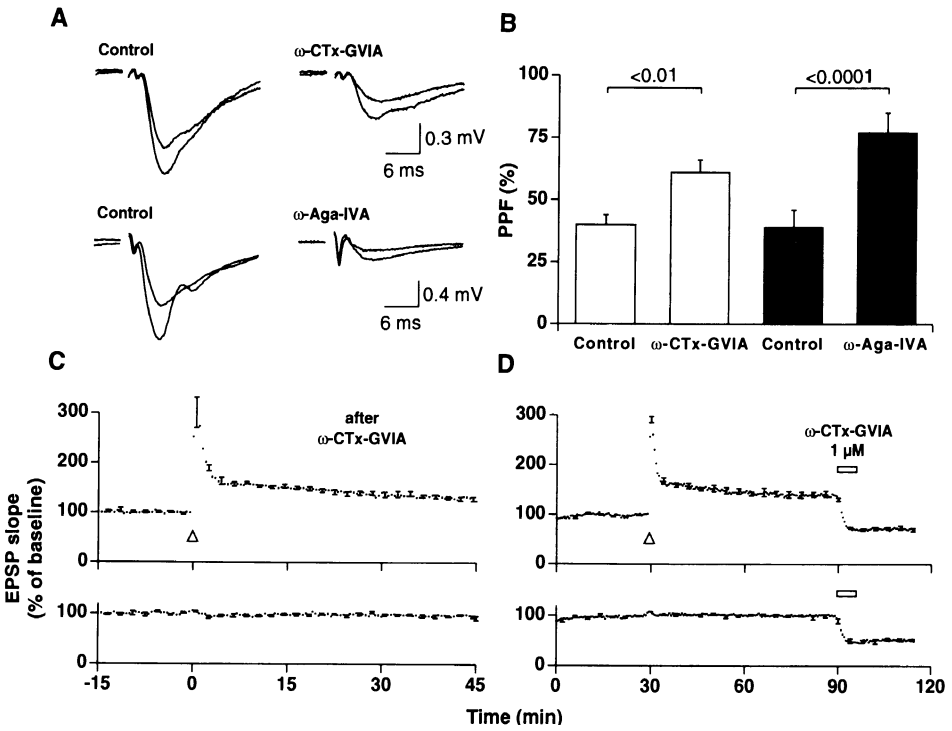

Fig. 4. Short- and long-term plasticity of synaptic transmission supported by Q-type Ca^{2+} channels. (**A**) Effect of ω-CTx-GVIA or ω-Aga-IVA on PPF. In each panel, the response to the first stimulus is smaller than the response to the second stimulus applied 40 ms later. Individual traces are averages of five consecutive responses. Top row, PPF before (left) or after (right) blockade of N-type channels with 1 μM ω-CTx-GVIA in a representative experiment; bottom row, PPF before (left) or after (right) inhibition of Q-type channels with 1 μM ω-Aga-IVA. (**B**) Mean ± SEM of the degree of PPF (percent increase in the maximal slope of the second response relative to that of the first response), before and after application of ω-CTx-GVIA ($n = 14$) or before and after application of ω-Aga-IVA ($n = 5$). (**C**) Induction and expression of LTP after application of 1 μM ω-CTx-GVIA. Ensemble average of EPSP slope, normalized to baseline values before tetanic stimulation (△, 100 Hz for 1 s in two trains separated by 30 s). Upper panel, responses in the pathways that were tetanized (38 ± 1% potentiation above baseline at 30 min post-tetanus, $n = 5$); lower panel, responses to stimulation of separate pathways in the same slices that did not undergo tetanic stimulation ($n = 5$). (**D**) Pooled data from experiments in which ω-CTx-GVIA was applied 60 min after induction of LTP by tetanus (△). In both potentiated (upper) and control (lower) pathways, the depression caused by ω-CTx-GVIA was 49 ± 2% ($n = 4$).

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 mGlu, $GABA_B$, 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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15. The lack of effect of low doses of ω-Aga-IVA is probably not the result of poor penetration of the slice; comparably low concentrations of ω-CTx-GVIA act potently and quickly.
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19. The ω-Aga-IVA blocks Q-type Ca^{2+} channels by 50% at ~200 nM (*4, 6*) but has no effect on N-type channels even at ~1 μM (*14, 18*).
20. For example, ω-Aga-IVA reduces synaptic transmission at mouse endplates by 50% at ~20 nM (Y. I. Kim, J. M. Longacher, M. P. Viglione, *Biophys. J.*, in press). Similarly, ω-Aga-IVA reduces inhibitory transmission onto deep cerebellar neurons with an IC_{50} value (amount of a substance required to inhibit activity by 50%) of 9.4 nM and decreases dopamine release from striatal synaptosomes with an IC_{50} of ~30 nM (*31*).
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25. Our results do not rule out the possibility that modulation of transmitter release occurs independently of Ca^{2+} channels, perhaps by altering the Ca^{2+} dependence of secretion [K. P. Scholz and R. J. Miller, *ibid.* **8**, 1139 (1992)].
26. The increase in PPF after N-type channel blockade appears not to be an effect of diminished EPSP amplitude. When stimulus strength was decreased, reducing EPSP amplitude to the same degree as that produced by ω-CTx-GVIA, PPF was only 43 ± 2%, significantly less than the 66 ± 5% PPF found with blockade of N-type channels [ω-CTx-GVIA versus size-matched control, paired $t(6) = 3.358$, $P < 0.05$]. It seems probable, therefore, that the effect of the toxins on PPF is a direct consequence of the reduction in Ca^{2+} influx during the action potential. This was supported by the observed effect of lowering extracellular Ca^{2+}, which also significantly enhanced PPF [35 ± 3% in 2.0 mM Ca^{2+} compared with 75 ± 4% in 1.3 mM Ca^{2+}, paired $t(6) = 15.0$, $P < 0.0001$]. Additionally, blockade of N-type Ca^{2+} channels caused no obvious change in the dependence of PPF on the interstimulus interval.
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33. The fractional release in the presence of toxin relative to control (R) should be proportional to the nth power of Ca^{2+} entry near release sites, where n is ≥2 (*32*). If Q- and N-type channels contribute fractions q and $1 - q$, respectively, of the total Ca^{2+} entry in the absence of toxin, their contributions in the presence of toxin will be $f_Q q + f_N(1 - q)$, where f_Q and f_N are the fractions of Q- and N-type Ca^{2+} channels remaining unblocked. Then $R = [f_Q q + f_N(1 - q)]^n$. Taking $n = 2$ as a conservative estimate, and bearing in mind that 1 μM ω-CTx-GVIA completely blocks N-type but spares Q-type Ca^{2+} channels, $R = [q]^2 = 0.54$, and $q = 0.735$ (q would be as high as 0.814 if $n = 3$). The proportion of transmission that would be left after complete and selective blockade of Q-type channels would then be estimated as $R = [1 - q]^n = (0.265)^2 = 0.070$. The expected effect of block of Q-type channels by 1 μM ω-Aga-IVA can be estimated by noting that ω-Aga-IVA blocks Q-type current in oocytes with half-maximal block at ~200 nM (*4*). Thus, $f_Q = 0.167$, and $R = [0.167q + (1 - q)]^n = 0.15$, in good agreement with the fraction of transmission remaining after ω-Aga-IVA.
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