valuable suggestions by J. Vaupel. Supported by the National Institute on Aging (grant AG08761-01) and a grant from the University of California, Davis, Collaborative Research Program.

7 April 1994; accepted 11 July 1994

Response: The analysis by Vaupel *et al.* allows the extension of our data in ways that we (*1*) did not originally envision and provides a much better fit of the raw data. The slope of the Gompertz curve changes at 8 days of age, but mortality still continues to increase exponentially until the end of life. However, large errors in the estimation of mortality rates late in life, which result from the small number of deaths during this period, prevent us from determining the accuracy of this statement with a high degree of precision.

I find the statements from Curtsinger *et al.* to be convincing, and these precautions seem to adequately rule out the possibility that there was significant contamination by progeny in the aging cohorts displayed in their earlier report (*2*). Nevertheless, any significant amount of progeny contamination can produce a huge artifact when only a small minority of the starting population are being examined as is done in examining the oldest old. Great care must be taken to avoid it.

The analyses conducted by Wang *et al.* purport to show (i) that a "nonparametric locally weighted least squares method" provides a better fit (*3*) and detects a decrease in mortality rates at "between 8 and 10 days" and (ii) that each of the 79 genotypes analyzed in our report (*1*) themselves appear to be composed of two slopes. We completely concur on the first aspect of their analysis (*4*). However, we are uncertain that the deviations from the exponential curve are biologically significant. It remains to be demonstrated that the details of these deviations from the Gompertz will be replicated in further analyses. It is our opinion that experimental replication, not extensive mathematical analyses, should be the critical basis for testing theories in this area.

We disagree on the second aspect of the comment by Wang *et al.* The analyses have a high inherent inaccuracy because the analysis is based on total cohorts of 30 animals or less. Many of the individual estimates of age-specific rates of mortality are based on none or only a single death in a given time period. However, too few details are presented to allow us to determine the accuracy of the methodology. We do agree that there was censoring of the data [this was stated in the original report (*1*)] and that this censoring has resulted in apparently higher mortality rates early in life. In analyzing this data, Wang *et al.* suggest that the four quartiles behave synchronously; because the division into quartiles was based on mean life span (*1*), it is not unexpected that there is a fairly uniform distribution of mean life span in each quartile. However, the mean life spans of these RIs are distributed essentially normally (*5*, *6*) and all RIs are derived from crosses between the Bristol and Bergerac wild-type strains of *C. elegans* (*5*, *6*, *7*).

The principal problem with the analyses put forth by Wang *et al.* is twofold. First, the small population size results in considerable inaccuracy in the estimates of mortality rates and the rates estimated could be off by several orders of magnitude. Second, Wang *et al.* found absolute mortality rates early in life to range from 10^{-8} to 10^{-6}, which can be compared with our estimates of 10^{-3} or less (*1*, *5*, *8*) in populations of about 200 worms. Error estimates in the latter two studies (*5*, *8*) were obtained directly by analysis of the four component populations, each of 50 worms, and direct estimation of error. The standard error of the mortality rate at 3 days of age in these estimates (*8*) was 15 to 40% of the mean. Also, the log mortality rate at 3 days of age in two different estimates for the wild type (N2) were −2.09 (*5*) and −2.70 (*8*), which suggests considerable variation between experiments. In contrast, Wang *et al.* suggest that less than one worm out of 100 million is dying per day early in life; surely an estimate such as this cannot be made on a cohort of population size 30.

Before the methods proposed by Wang *et al.* are accepted for the analysis of mortality in small cohorts, they should show that their procedure allows the accurate reconstruction of the original mortality rates with the use of small, simulated data sets. One could simulate a population of organisms dying with exponential kinetics to see if their analytic method would generate an exponential model after sampling populations of size 30. Indeed, one of the principal arguments put forth in an earlier study "is that it may not be possible to determine the mortality pattern of a species from data on 100 or even fewer individuals . . ." (*3*, p. 460).

Thomas E. Johnson
Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309–0447, USA

REFERENCES

1. A. Brooks, G. J. Lithgow, T. E. Johnson, *Science* **263**, 668 (1994).
2. J. W. Curtsinger, H. H. Fukui, D. R. Townsend, J. W. Vaupel, *ibid.* **258**, 461 (1992).
3. J. R. Carey, P. Liedo, D. Orozco, J. W. Vaupel, *ibid.*, p. 457.
4. J. W. Vaupel, T. E. Johnson, G. J. Lithgow, *ibid.* **266**, 826 (1994).
5. T. E. Johnson, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3777 (1987).
6. A. Brooks, thesis, University of Colorado, (1993).
7. ——— and T. E. Johnson, *Heredity* **67**, 19 (1991).
8. T. E. Johnson, *Science* **249**, 908 (1990).

18 May 1994; accepted 11 July 1994

Identification of Calcium Channels That Control Neurosecretion

The report by David B. Wheeler *et al.* (*1*) addresses the important question of which Ca^{2+} channel types control synaptic transmission in the mammalian central nervous system. Wheeler *et al.* studied glutamatergic transmission between Schaffer collateral fibers and CA1 pyramidal neurons in the rat hippocampus and used synthetic toxins that target high voltage–activated Ca^{2+} channels in an effort to identify which types trigger glutamate release at this synapse. Wheeler *et al.* argue for the primary involvement of a novel class of Ca^{2+} channel, which they have labeled "Q." In pharmacological experiments such as these, three criteria should be met before conclusions can be drawn with confidence: (i) the concentration of antagonists at the synaptic site must be known, (ii) estimates of potency must be made at or near equilibrium for antagonist binding, and (iii) the antagonists employed should be specific. These fundamental criteria have not been consistently met in the report by Wheeler *et al.*

Wheeler *et al.*, measuring the field excitatory postsynaptic potential (fEPSP), demonstrate a slow onset for action of ω-agatoxin IVA [IVA, a P channel antagonist (*2*)]: 30 nM toxin produced no effect in 20 min and 200 nM produced inhibition at a rate of about 1% per minute. They use this to argue for the relative inefficacy of IVA on Schaffer collateral Ca^{2+} channels (and for the lack of P channels in Schaffer collateral nerve terminals). Our recent results suggest that the rate of onset for toxin action in a tissue slice is largely a function of the rate of toxin delivery to the synaptic region rather than the on-rate for toxin binding. The application times that Wheeler *et al.* used in their experiments with 30 nM IVA, therefore, are insufficient to achieve a steady-state concentration in the synaptic region. With the use of whole cell recording from superficial CA1 neurons in the slice, we found that 100 to 200 nM IVA

eliminated the excitatory postsynaptic current (EPSC) in about 10 to 20 min (*3*). To test whether the slower rate of blockade observed by Wheeler *et al.* might be a result of a slow delivery of the toxin deep within the tissue slice (where field recordings are performed), we studied the action of IVA on EPSCs and fEPSPs recorded simultaneously in the slice. IVA acted more quickly and was more efficacious on the EPSC as compared with the fEPSP (Fig. 1). This result points to uncertainties with regard to toxin concentration in the slice and suggests that barriers to IVA diffusion (or nonspecific adsorption of the toxin) lead to concentration gradients in the tissue.

When sufficient time is provided for antagonist binding to approach equilibrium, more reliable estimates of toxin potency can be made. In our recent experiments, we have found (*4*) that inhibition of synaptic transmission produced by bath application of 30 nM IVA (in 1 mg/ml bovine serum albumin to minimize toxin adsorption) reached a steady-state in 31.5 ± 3 min and produced 81.8 ± 6.7% reduction of EPSC amplitude (n = 5). This suggests an IC_{50} (the amount of IVA required to inhibit the EPSC by 50%) action at Schaffer collateral nerve terminals below 30 nM. This value is in close agreement with other reports on IC_{50} values for IVA-induced blockade of nerve terminal Ca^{2+} channels determined with the use of diverse experimental techniques and preparations. Purified IVA inhibits depolarization-induced $^{45}Ca^{2+}$ influx into rat brain synaptosomes with an IC_{50} of about 20 nM (*2*). Similarly, our experiments with rat cortical synaptosomes (under near equilibrium conditions for IVA binding) demonstrate an IC_{50} of about 30 nM for inhibition of glutamate release (*5*). Takahashi and Momiyama (*6*), using perfusion buffers containing 1.0 mg per ml cytochrome c, report an IC_{50} of about 10 nM for IVA block of inhibitory synaptic transmission in the cerebellum. The apparent potency of IVA in blocking presynaptic Ca^{2+} channels is less than that found for its inhibition of isolated Purkinje neuron somatic Ca^{2+} currents, where an affinity of 1 to 3 nM has been reported (*7*, *8*). Different preparations of synthetic IVA are likely to have different potencies, however (*9*).

Toxin specificity is also of concern in the experiments of Wheeler *et al.* They argue for the existence of a new channel type based on the sensitivity of Schaffer collateral–CA1 synaptic transmission to ω-conotoxin MVIIC (ω-CTx-MVIIC). This toxin cannot be used as a discriminator of high voltage–activated Ca^{2+} channel types in synaptic transmission because it is largely nonspecific, blocking both N and P currents (*10*, *11*) as well as current expressed in *Xenopus* oocytes injected with cRNA encoding the α_{1A}, α_2/δ, and β subunits of the Ca^{2+} channel (*12*). Differential potency among the channel types has not been established. Recent preliminary data from Swartz *et al.* (*11*) suggest that the affinities of ω-CTx-MVIIC at N- and P-type channels are similar and that blockade occurs with a K_i of about 10 nM. Elimination of Schaffer collateral–CA1 synaptic transmission with 1.5 to 5 μM ω-CTx-MVIIC as shown by Wheeler *et al.* would, thus, be expected regardless of the high voltage–activated Ca^{2+} channel involved.

One final issue should be considered. The use of a complex physiological process (such as neurosecretion) as an assay for block of presynaptic calcium channels would result in an underestimation of potency if there are "spare channels" at the presynaptic terminal. Vertebrate smooth muscle provides an excellent example of such a phenomenon; blockade of more than 90% of cholinergic receptors by irreversible antagonists is necessary to produce a measurable inhibition of agonist-induced contraction (*13*). The dissociation constant of antagonist for receptor, therefore, is much less than its IC_{50} for inhibition of contraction. By analogy, if Ca^{2+} channels are present at a density in excess of that necessary to produce maximal secretion, blockade of 50% of the channels will produce less than 50% reduction in secretion—and, thus, would lead to underestimates of potency for IVA at the nerve terminal Ca^{2+} channels. Our studies of transmitter release lend credibility to this notion of spare channels. We have found that at least two types of Ca^{2+} channel coexist in nerve terminals. By blocking one type with a selective antagonist, the other type, when strongly activated, can support maximal secretion (*5*, *14*). Thus, until the quantitative relationship between Ca^{2+} entry and exocytosis is established, the differences in potency reported for IVA's inhibition of synaptic transmission compared with its blockade of Ca^{2+} currents should not be taken as evidence supporting the involvement of a Ca^{2+} channel α_1 subunit distinct from that native to Purkinje neuron cell bodies. If differences in toxin affinity in diverse systems are established, it will be important to discriminate between several possible underlying causes, including distinct Ca^{2+} channel α_1 subunits, differences in multisubunit channel composition, and alterations in posttranslational modification of the channel complex.

A premature subdivision of the classification scheme of subtypes of IVA-sensitive current, based on incomplete pharmacological information, should be avoided at this point. Definitive proof of novel channels responsible for Ca^{2+} influx and exocytosis in mammalian nerve terminals awaits the investigation of a more complete battery of specific antagonists, more appropriate experimental design, and more systematic pharmacological characterization of cloned Ca^{2+} channels expressed in heterogeneous environments.

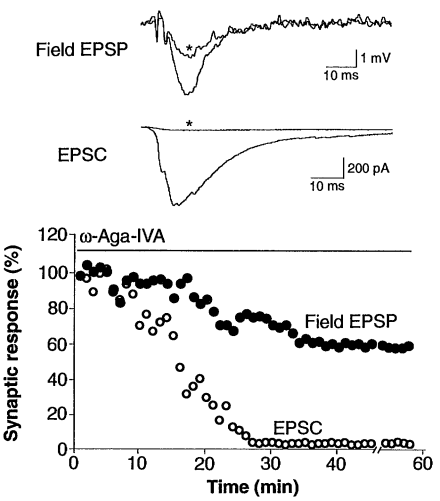

Fig. 1. Latency to onset and efficacy of ω-Aga-IVA in relation to recording configuration. Hippocampal slices (400 μm) were prepared with standard methods. The Stratum radiatum was stimulated with the use of an extracellular, bipolar electrode. Synaptic currents were recorded from CA1 pyramidal neurons near the surface of the slice with tight seal, whole cell recording methods. Field EPSPs were simultaneously recorded with an extracellular electrode placed deep in the radiatum, adjacent to the intracellular recording electrode. Upper panels show fEPSPs and EPSCs recorded prior to and 60 or 30 min, respectively, after (*) application of 200 nM ω-Aga-IVA. Lower panel shows the complete time course for IVA's action in a different experiment. Time of IVA application is denoted by the horizontal bar.

Kathleen Dunlap
Jennifer I. Luebke
Timothy J. Turner
Department of Physiology,
Tufts University School of Medicine,
Boston, MA 02111, USA

REFERENCES AND NOTES

1. D. B. Wheeler, A. Randall, R. W. Tsien, *Science* **264**, 107 (1994).
2. I. M. Mintz *et al.*, *Nature* **355**, 827 (1992).
3. J. I. Luebke, K. Dunlap, T. J. Turner, *Neuron* **11**, 895 (1993).
4. J. I. Luebke and K. Dunlap, unpublished observations.
5. T. J. Turner, M. E. Adams, K. Dunlap, *Science* **258**, 310 (1992).
6. T. Takahashi and A. Momiyama, *Nature* **366**, 156 (1993); P. E. Castillo, M. G. Weisskopf, R. A. Nicoll, *Neuron* **12**, 261 (1994); W. G. Regher and I. M. Mintz, *ibid.*, p. 605.
7. I. M. Mintz and B. P. Bean, *Neuropharmacol.* **32**, 1161 (1993).
8. I. M. Mintz, M. E. Adams, B. P. Bean, *Neuron* **9**, 85 (1992).
9. A. Ganong, Pfizer Pharmaceuticals, personal communication.
10. D. R. Hillyard *et al.*, *Neuron* **9**, 69 (1992).

11. K. J. Swartz, I. M. Mintz, L. M. Boland, B. P. Bean, *Soc. Neurosci. Abstr.* **19**, 1478 (1993).
12. W. A. Sather *et al.*, *Neuron* **11**, 291 (1993).
13. R. F. Furchgott, in *Advances in Drug Research* (Academic Press, New York, 1966), p. 21.
14. T. J. Turner, M. E. Adams, K. Dunlap, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 9518 (1993).

20 June 1994; accepted 4 October 1994

Response: We welcome the opportunity to amplify on experimental and interpretive aspects of our study that could not be accomodated within a brief report (*1*). We agree with Dunlap *et al.* that pharmacological experiments of synaptic transmissions should be interpreted with caution, particularly with respect to issues of drug penetration and efficacy in brain slices. We disagree, however, with several of their contentions about our report. We find no evidence for a maintained gradient of ω-Aga-IVA concentration within the slice. The observed response of neurotransmission to ω-Aga-IVA (*1*–*5*) is consistent with participation of Q-type channels. The statements by Dunlap *et al.* about the degree of specificity of ω-CTx-MVIIC seem to miss the point of our experiments (*1*), which tested for a possible role of R-type channels (*6*, *7*). Their arguments regarding "spare channels" contradict published evidence for a power-law relationship between Ca^{2+} influx and action potential–triggered transmitter release (*1*–*5*). Upon review of all of the available evidence, we stand by our conclusion that synaptic transmission at hippocampal CA3-CA1 synapses is supported, in large part, by voltage-gated Ca^{2+} entry with the pharmacological profile of Q-type channels (*7*, *8*).

One of the main contentions of Dunlap *et al.* is that a gradient of toxin concentration can exist in the steady-state, preventing penetration of toxin to the depths of the hippocampal slice. Their evidence for this rests on a discrepancy between the degree to which ω-Aga-IVA inhibits fEPSPs monitored with fEPSP and intracellular EPSCs measured with whole cell patch-clamps. Their figure 1 shows steady but only partial block of the fEPSP at a time when the EPSC has been abolished, which they interpret as implying a standing gradient of ω-Aga-IVA. In contrast, we find good agreement between the fEPSP and the EPSC in the rate and degree of their inhibition by ω-Aga-IVA. In representative recordings of fEPSP and EPSC (Fig. 1A), evoked by electrical stimulation of the Schaffer collateral–commisural pathway ω-Aga-IVA (200 nM), both indices of the synaptic response are reduced in a parallel fashion (n = 7, Fig. 1B). There is more variability among the individual whole cell recordings, as befits responses from individual cells, but overall, the inhibition develops with a similar time constant (τ_{fEPSP} = 14.3 min, τ_{EPSC} = 15.2 min). The transmission remaining in the steady-state is similar for the fEPSP and the EPSC (17 ± 2 and 15 ± 1%, respectively; $P > 0.16$ by paired Student's t test). Thus, in our hands, field potentials and whole cell currents give roughly equivalent measures of ω-Aga-IVA action. It is reassuring to find such agreement, as each of these approaches has different strengths and limitations. Field recordings have their own advantages: They are relatively noninvasive, less prone to cell run-down than whole cell recordings, and provide an average measure of the activity of synapses on many postsynaptic cells in a region of the slice that is less prone to mechanical damage from the slicing procedure.

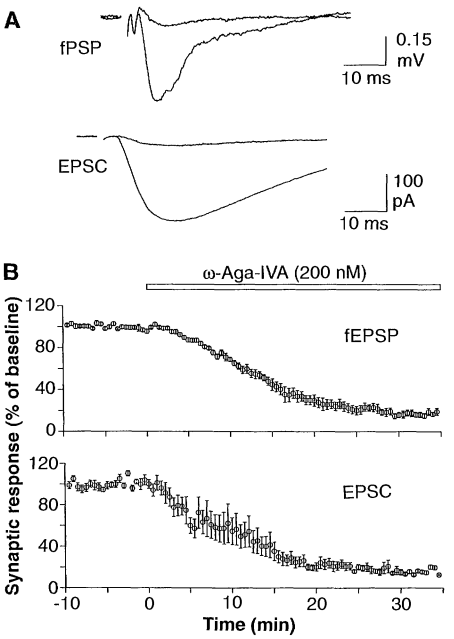

Fig. 1. Effect of ω-Aga-IVA on the strength of synaptic transmission. (**A**) Representative examples of simultaneous recordings of the fEPSP and the EPSC evoked at 0.1 Hz. Sweeps are averages of 30 consecutive responses, collected from −5 to 0 min (larger amplitudes) and from 30 to 35 min after beginning application of ω-Aga-IVA (smaller amplitudes). (**B**) Average responses of fEPSP slope and EPSC amplitude from seven experiments like that in (A). Responses were taken as the average of three consecutive signals and were normalized to their baseline value (average of determinations during the 10 min preceding addition of ω-Aga-IVA). Symbols represent the mean ± SEM of these responses, averaged across the seven experiments. All experiments were carried out in the continuous presence of cytochrome c (0.1 mg/ml) to saturate nonspecific protein binding sites and picrotoxin (5 μM) to reduce contamination of the EPSC by the GABA-containing inhibitory postsynaptic current. In order to test the hypotheis of Dunlap *et al.* as fairly as possible, the fEPSP was recorded with the electrode tip positioned about 200 μm into the slice, while the EPSC was recorded from the most superficial cell detectably contacted by the patch pipette.

To gain additional information about how peptide toxins behave in slices, we carried out experiments with ω-CTx-GVIA (*9*), which is similar to ω-Aga-IVA in size and overall structure. We find that ω-CTx-GVIA is completely efficacious, even at low concentrations. Whereas the rate of block of the fEPSP increased with ω-CTx-GVIA concentration; as expected, a similar degree of inhibition (~45%) (*1*) was achieved during application of 10 nM, 30 nM, or 3 μM ω-CTx-GVIA. This result supports our contention that peptide toxin molecules can gain access to all the Ca^{2+} channels involved in synaptic transmission. At any given concentration of ω-CTx-GVIA, the rate of block is about fivefold slower than that seen for N-type current in dissociated rat sympathetic neurons (*10*). A slowing of toxin equilibration would be predicted from studies of the diffusion of marker molecules within slices (*11*). Our experiments with ω-Aga-IVA and ω-CTx-GVIA are consistent with the idea that the diffusion of peptide toxins within slices is retarded but not prevented.

How do the experimental results from slice recordings compare with predictions for the dose dependence of toxin-block of neurotransmission based on Ca^{2+} channel pharmacology? Dose-response curves (Fig. 2) can be calculated by presuming that P-type channels or Q-type channels are the dominant Ca^{2+} entry pathway, along with a fixed and smaller contribution of N-type channels (*1*, *2*, *4*, *5*). The IC_{50} values for ω-Aga-IVA block were taken as 1 nM for P-type channels (*12*) and 89 nM for Q-type channels (*7*). These values were translated into predictions for hippocampal neurotransmission (Fig. 2) on the basis of experimental evidence for a power-law relationship (n = 3 to 4) between Ca^{2+} entry and neurotransmitter release (inset) (*5*, *13*). The predicted IC_{50} values for ω-Aga-IVA block of transmitter release are in the range of 0.3 nM for synapses dominated by P-type channels and 20 nM for synapses dominated by Q-type channels. These predictions are compared (Fig. 2) with experimental data obtained from recordings of excitatory transmission in hippocampal slices (*1*, *2*–*4*, *14*), which include a new set of data we obtained. The synapses show significant inhibition with 45 min exposures to 30 nM ω-Aga-IVA (n = 9). The data appear clustered around the curve predicted for transmission mediated by Q-type channels.

Dunlap and colleagues state that Ca^{2+} entry is sufficient to saturate neurosecretion, as if there were "spare channels." This stands in contrast to studies in the hippocampus (*13*), which include direct evidence from intracellular Ca^{2+} measurements (*5*). These studies demonstrate that *action potential*–triggered neurotransmission

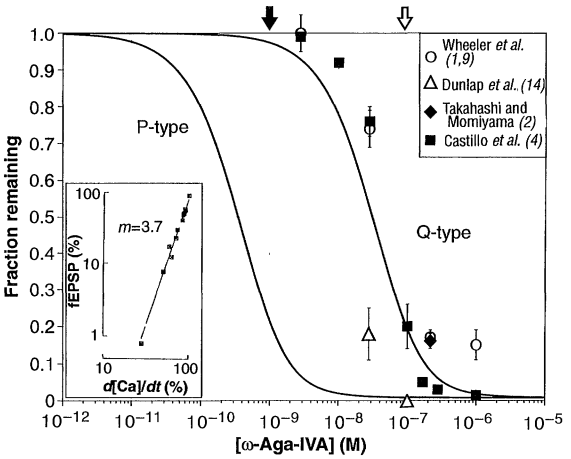

Fig. 2. Comparison between experimentally determined block of neurotransmission by ω-Aga-IVA and hypothetical dose-response relationships. Symbols are the mean ± SEM of measures of excitatory hippocampal synaptic transmission in the presence of ω-Aga-IVA, taken from (*1*) and (*9*) (○), (*14*) (△), (*2*) (◆), and (*4*) (■). For additional data from cerebellum, see (*2, 18*). The curves were generated with the use of the general equation:

$$\text{Fraction remaining} = [P_{GVIA} + P_{Aga}/(1+[\omega\text{-Aga-IVA}]/K_D)]^n \quad (1)$$

where p_{Aga} is the estimated fractional contribution of ω-Aga-IVA–sensitive channels (0.8) and P_{GVIA} is the estimated fractional contribution of ω-CTx-GVIA–sensitive channels (0.2) (*1*), K_D is the IC_{50} for ω-Aga-IVA block of P-type channels (1 nM, solid vertical arrow, (*12*) or Q-type channels [89 nM, open vertical arrow, (*7*)]. The exponent of $n = 3$ was chosen as a conservative estimate. Experimental determination of the power-law relationship between relative Ca^{2+} influx (monitored with fura-2) and fEPSP slope (Inset) yields an exponent of 3.7. Inset reproduced from (*5*) with permission.

at hippocampal CA3-CA1 synapses obeys a classical power-law in its dependence on Ca^{2+} entry (exponent = 3.7, Fig. 2 inset), in accord with the classic description by Dodge and Rahamimoff of Ca^{2+} entry and neurotranmission at the neuromuscular junction (*16*). The application by Dunlap *et al.* of the concept of "spare channels" to action potential–triggered neurotransmission contradicts the results of several groups (*1, 2, 4, 5*), including their own (*3*); Percentage of transmission blocked by ω-Aga-IVA, and percentage blocked by ω-CTx-GVIA, add up to more than 100%, even though the toxins are known to act on separate and nonoverlapping populations of channels (*12*). This can be readily explained in terms of a power-law relationship, but is not expected for the "spare channel" hypothesis.

The concept of "spare channels" may be appropriate when considering neurotransmitter release triggered by high K^+ depolarizations in synaptosomes (*16*). In this case, blockage of either N-type channels or ω-Aga-IVA–sensitive channels has little or no effect on transmitter release, even though the combined effect of both agents is quite significant. This pattern of behavior is different from that of action potential–triggered neurotransmission in slices or even synaptosomes stimulated with lower amounts of K^+ (*16*). The difference may be accounted for by the way that Ca^{2+} channels respond to different patterns of depolarization (*17*). During a normal action potential, the chances are small that any single Ca^{2+} channel will open for long enough to trigger transmitter release on its own; thus, the participation of multiple channels greatly promotes neurotransmission. In contrast, prolonged and strong depolarizations dramatically increase channel opening probability and the likelihood that Ca^{2+} influx through an individual channel will be sufficient to trigger release by a nearby vesicle. This lessens the dependence of transmitter release on multiple channels. In support of this explanation, we find that the impact of blocking N-type channels on neurotransmission is greatly attentuated by prolonging the action potential with 4-aminopyridine (*17*). Thus, the degree to which different Ca^{2+} channel types participate in neurotransmissions can be radically altered by varying the duration of the depolarizing stimulus.

Dunlap *et al.* raise the issue of the specificity of toxins and their usefulness in channel identification with regard to our use of ω-CTx-MVIIC. We stated explicitly in our report (*1*) that ω-CTx-MVIIC blocks both N- and Q-type channels and thus cannot be used on its own to establish whether or not Q-type channels are involved (*1*). As we indicated, ω-CTx-MVIIC was used because it spares R-type channels (*6, 7*); if R-type channels had made a substantial contribution, some synaptic transmission would be spared by ω-CTx-MVIIC. Therefore, it is incorrect for Dunlap *et al.* to state that blockage by this toxin "would, thus, be expected regardless of the high voltage–activated channel involved." When used in conjunction with ω-CTx-GVIA and low concentrations of ω-Aga-IVA, ω-CTx-MVIIC also provides useful corroboration of the possible involvement of Q-type channels.

Dunlap *et al.* cite studies from others (*2, 4, 18*) as providing "a preponderance of evidence" for the involvement of P-type channels. In reporting that ω-Aga-IVA reduced synaptic transmission, each of these groups discussed possible involvement of P-type channels, but was also careful to qualify their conclusions by explicitly allowing for the participation of other ω-Aga-IVA–sensitive currents (*7–9*). In earlier studies of ω-Aga-IVA action in other systems [for example, (*19*)], P-type channels were invoked by default as the only Ca^{2+} channel known to respond to ω-Aga-IVA. It will be interesting to reexamine these systems in light of recent information about the properties of P- and Q-type channels (*7–9*) and expectations as to how their contribution to neurotransmission would be reflected in the response to ω-Aga-IVA (Fig. 2).

David B. Wheeler
Richard W. Tsien
Department of Molecular and Cellular Physiology, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305, USA
Andrew Randall
Neurobiology Division, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, United Kingdom

REFERENCES AND NOTES

1. D. B. Wheeler, A. Randall, R. W. Tsien, *Science* **264**, 107 (1994).
2. T. Takahashi and A. Momiyama, *Nature* **366**, 156 (1993).
3. J. I. Luebke, K. Dunlap, T. J. Turner, *Neuron* **11**, 895 (1993).
4. P. B. Castillo, M. G. Weisskopf, R. A. Nicoll, *ibid.* **12**, 261 (1994).
5. L. G. Wu and P. Saggau, *J. Neurosci.* **14**, 645 (1994).
6. J.-F. Zhang *et al.*, *Neuropharmacology* **32**, 1075 (1993).
7. A. Randall and R. W. Tsien, in preparation.
8. W. A. Sather *et al.*, *Neuron* **11**, 291 (1993); A. Stea *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, in press.
9. D. B. Wheeler, A. Randall, R. W. Tsien, unpublished observations.
10. L. M. Boland, J. A. Morrill, B. P. Ben, *J. Neurosci.*, in press.
11. H-G. Lipinski and D. Bingmann, *Brain Res.* **437**, 26 (1987); C. J. McBain, S. F. Traynelis, R. Dingledine, *Science* **249**, 674 (1990).
12. I. M. Mintz, M. E. Adams, B. P. Bean, *Neuron.* **9**, 85 (1992); I. M. Mintz and B. P. Bean, *Neuropharmacology* **32**, 1161 (1993).
13. R. Dingledine and G. Somjen, *Brain Res.* **207**, 218 (1981); D. Mulkeen, R. Anwyl, M. Rowan, *ibid.* **447**, 234 (1988).
14. K. Dunlap, J. I. Luebke, T. J. Turner, *Science* **266**, 828 (1994).
15. F. A. Dodge and R. Rahaminoff, *J. Physiol.* **193**, 419 (1967).
16. T. J. Turner, M. E. Adams, K. Dunlap, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 9518 (1993).
17. D. B. Wheeler, A. D. Randall, R. W. Tsien, *J. Physiol.*, in press.
18. W. D. Regehr and I. M. Mintz, *Neuron* **12**, 605 (1994).
19. D. A. Protti and O. D. Uchitel, *NeuroReport* **5**, 333 (1993).
20. D.B.W. is a Howard Hughes Medical Institute Predoctoral Fellow and A.R. was a Lucille P. Markey Visiting Scholar. We thank R. W. Aldrich, K. Deisseroth, W. A. Sather, and J.-F. Zhang for helpful discussions in the preparation of this manuscript. Supported by an NIMH–Silvio Conte Center for Neuroscience Research Award.

19 August 1994; accepted 4 October 1994