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Quantal Release of Transmitter Is Not Associated with Channel Opening on the Neuronal Membrane

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The traditional view that quantal release of neurotransmitter results from the fusion of transmitter-containing vesicles with the neuronal membrane has been recently challenged. Although various alternative mechanisms have been proposed, a common element among them is the release of cytoplasmic transmitter, which, in one view, could occur through large conductance channels on the presynaptic membrane. Six nerve-muscle cell pairs were examined with a whole-cell patch clamp for the presence of such channels that are associated with the production of miniature end-plate potentials. Examination of the neuronal membrane current during the occurrence of 822 miniature end-plate potentials produced no evidence of large channels. Thus it is unlikely that quantal release is mediated by such channels in the neuromuscular junction.

FOR A NUMBER OF YEARS, QUANTAL release of neurotransmitter has been understood to occur through fusion of small (50 nm in diameter) transmitter-containing vesicles with the presynaptic membrane. Recent challenges to this explanation of quantal release consider the direct translocation of cytoplasmic transmitter to the extracellular region (*1*). One possible mechanism for such a translocation is via channels within the presynaptic membrane that open to "gate" the cytoplasmic transmitter. We have looked for the presence of such channels in the nerve membrane of nerve-muscle cell pairs produced from *Xenopus* nerve and muscle cells in culture.

The use of cultured cells of *Xenopus* has many advantages for this study. (i) Isolated nerve and muscle cells can be manipulated into contact with each other, and quantal release of transmitter can be detected within minutes of contact, from both the neuronal soma and the neurites (*2*). (ii) The approximately spherical soma may be placed under a whole-cell patch clamp, and the resting membrane impedance is high (>200 megohms) so that background current noise is low (<2.5 pA at 1 kHz). (iii) The transmitter is acetylcholine (ACh), which has a high enough molecular weight (AChCl, 181.7) that it will not pass through the large conductance (60 pS) ACh-receptor channel (*3*). Therefore, any channel that conducts ACh must be at least this large, which would make it detectable by our technique.

Xenopus cell cultures were prepared as described (*2*). Before the start of the recording period, the culture medium was replaced by external solution (125 m*M* NaCl, 2 m*M* KCl, 10 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 20 m*M* Hepes, at *p*H 7.8). An isolated spherical muscle cell was impaled with a microelectrode (resting potential, −60 to −100 mV) and moved into contact with the soma of a neuron (Fig. 1). If the neuron was cholinergic, miniature end-plate potentials (MEPPs) were recorded from the muscle within 1 to 5 minutes. After this, the soma was placed under a voltage clamp with a patch pipette and a patch-clamp amplifier (List EPC-7). The internal solution was 92 m*M* KCl, 40 m*M* KOH, 1 m*M* $CaCl_2$, 11 m*M* EGTA, 1 m*M* $MgCl_2$, 20 m*M* Hepes, at *p*H 7.8. The soma membrane voltage was held at various potentials between −50 and +34 mV. Simultaneous recordings of the neuronal somata membrane current and muscle membrane potential were stored on magnetic tape (RACAL Store 4 FM) for later playback and analysis.

Recordings of 822 muscle MEPPs were made simultaneously with associated neuronal membrane currents resulting from six nerve-muscle contacts. Large neuronal membrane channels (currents >2.5 pA) were not seen immediately before, during, or after the peak of the MEPPs. This lack of neuronal channel opening was a consistent finding despite the presence of very large depolarizations of the muscle membrane (30 mV, which indicates a large amount of ACh was released). Records from three different nerve-muscle contacts are shown in Fig. 2A. The bottom set exhibits the highest resolution of neuronal currents (<2.5 pA), and no neuronal single channels were detected. Records of neuronal single-channel currents that occurred at random, not correlated with the MEPPs, illustrate the high current resolution of the preparation (Fig. 2B). It is unlikely that we have failed to detect channel openings as a result of the transmitter having been released far from the soma along newly sprouted neurites for two reasons. (i) At the end of an experiment, when the muscle was pulled away from the neuron, no such neurites were observed. (ii) Neurite growth is slow [0.25 μm/min (*4*)] compared to the time course of our experiments (less than 5 minutes of nerve-muscle contact) so that even if new sprouting occurred instantly after contact, the length would be less than 1.25 μm at the end of the experiment. Because the reversal potential of such putative transmitter channels is unknown, the holding potential of the neuron was

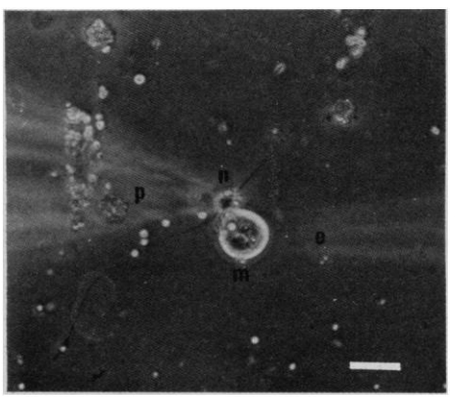

Fig. 1. Photomicrograph of manipulated nerve-muscle contacts in *Xenopus* cell culture. A spherical muscle cell (m) is impaled with a microelectrode (e) and manipulated into contact with a neuronal soma (n). After the appearance of MEPPs, the soma was placed under a whole-cell clamp by a patch electrode (p). Scale bar, 30 μm.

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varied to increase the ability of their detection. The neuronal holding potential was slowly swept from −50 to +34 mV, during which time MEPPs continued to be recorded at higher frequency. However, no MEPP-correlated single-channel currents were observed on the neuronal membrane.

Challenges to the idea of vesicular release of transmitter have supported the idea of the release of cytoplasmic transmitter. Using the favorable characteristics of cultured *Xenopus* nerve and muscle cells, we have examined a possible alternate mechanism, whereby cytoplasmic ACh is gated through channels in the neuronal membrane. We found no evidence for such channels, even when the amount of transmitter released was large. We would expect to observe such channels given the large size of the ACh molecule. ACh will not pass through the high conductance (60 pS) ACh-receptor channel, a channel whose permeability is relatively nonspecific to cations. This ACh-receptor channel is detectable in our system, but we detected no transmitter channel. The argument could be made that such a channel could be highly selective to the ACh cation, excluding other smaller and more abundant cations such as K^+ or Na^+, and thereby could have a much smaller conductance and escape detection.

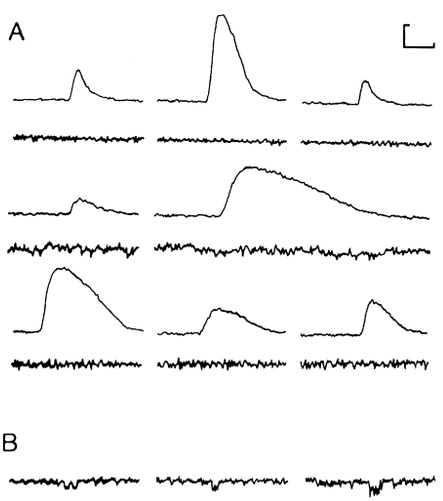

Fig. 2. (**A**) Simultaneous records of muscle membrane potentials and neuronal membrane currents during production of MEPPs. Each record consists of a pair of traces: the upper trace is muscle membrane potential, and the lower trace is neuronal membrane current. Records are presented from three separate nerve-muscle contacts. In no case were large single channels observed to open in the nerve membrane before, during, or after the MEPP. Scale bars, top: 5 mV, 25 pA, 20 msec; middle: 20 mV, 12.5 pA, 10 msec; bottom: 20 mV, 12.5 pA, 20 msec. (**B**) Single-channel openings on the neuronal membrane not correlated with MEPPs. These three channel openings occurred more than 200 msec before or after an MEPP on the muscle membrane. Scale bars: 12.5 pA; 20 msec, 10 msec, 10 msec.

To our knowledge, no channel with such very high selectivity to ACh has yet been described, although there is a report of a carrier, hexadecavalinomycin, that has a permeability to ACh that is 4.5 times as high as its permeability to K^+ (*5*).

Taken together, these arguments suggest that the amplitude of the current passing through presumptive neuronal membrane channels which regulate the release of ACh would be detectable by our technique, especially during the generation of a large MEPP. Thus, we conclude that it is very unlikely that such transmitter channels exist at the neuromuscular junction.

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The Structure of Sister Minichromosome DNA Before Anaphase in *Saccharomyces cerevisiae*

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The role of DNA topology in holding sister chromatids together before anaphase was investigated by analyzing the structure of a small circular minichromosome in cell cycle (cdc) mutants of the yeast *Saccharomyces cerevisiae*. In the majority of cells arrested after S phase but before anaphase, sister minichromosome molecules are not topologically interlocked with each other. The analysis of the ploidy of minichromosomes in cells that are released from arrest demonstrates that the sister molecules are properly segregated when the cell cycle block is removed. Therefore, sister minichromosome molecules need not remain topologically interlocked until anaphase in order to be properly segregated, and topological interlocking of sister DNA molecules apparently is not the primary force holding sister chromatids together.

SISTER CHROMATIDS ARE HELD TOgether from the time of their replication to the beginning of anaphase. This interaction is presumably essential to the mechanism that orients the segregation of two sister chromatids such that they segregate to opposite poles of the cell during anaphase. In addition, the ability of the cell to eliminate this interaction is essential for proper chromosome segregation in anaphase and in fact may be the primary event that initiates the onset of anaphase. Several models have invoked the topological interlocking of sister DNA molecules (by partially replicated centromere DNA or catenation) as the mechanism for holding sister chromatids together (*1–3*). In fact, at least some catenation of sister chromatids may exist before anaphase (*4, 5*). However, no one has determined the extent of topological interlocking between sister chromatids before anaphase or whether the persistence of these structures is essential for their proper segregation.

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To begin the analysis of the structure of chromosomal DNA at different stages of the cell cycle, we analyzed the structure of the minichromosome, pDK243, in an asynchronous population of wild-type yeast (*Saccharomyces cerevisiae*) cells grown at 23°C and 36°C (Fig. 1). This minichromosome is approximately 14 kb long and contains a putative origin of replication (*ARS1*), a centromere (*CEN3*), and a modified form of the *ADE3* gene (*ade3*-2p), which allows the ploidy of the minichromosome to be monitored in individual cells (*6*). A plasmid-specific probe identifies three distinct bands (Fig. 1a, wt at 36°C) that are present only in plasmid-bearing cells. The majority of pDK243 DNA in asynchronous wild-type cells (at either temperature) is closed circular monomer (Table 1) by the criterion that the major band comigrates with closed circular monomers from *Escherichia coli*. A small fraction of the minichromosome DNA isolated from these cells comigrates with nicked monomers from *E. coli*; the nicked circular form may be present inside the yeast cell or may be produced by nicking during the DNA isolation procedure. Approximately 15% of the total minichromosome DNA migrates at a third position, band C. The