Quantal Analysis of Excitatory Postsynaptic Potentials Induced in Hippocampal Neurons by Activation of Granule Cells*

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Summary. The values of quantal content (m) and quantal amplitude (q) of excitatory postsynaptic potentials (EPSPs) elicited in CA3 neurons by activation of granule cells were estimated in thin hippocampal sections maintained in vitro. For this purpose, DL-homocysteate was administered to granule cells, and trains of EPSPs that were typical for single granule cell activation were recorded from individual CA3 neurons. The amplitudes of the first and second EPSPs in each train were measured. From the mean and variance of the amplitudes of the EPSPs, the values of q and m were calculated. The values of m and q for the first EPSPs were estimated at 8.3 and 0.28 mV, respectively, on the average. Potentiation of the second EPSPs was accompanied by a two-fold increase in the values of m without changes in the values of q. Therefore, frequency potentiation in synapses between mossy fibers and CA3 neurons may be explained by an increase in number of released quanta. Amplitudes of EPSPs were found to fluctuate in a manner described by Poisson's law.

Key words: Hippocampus – Quantal amplitude – Quantal content – Mossy fiber – Frequency potentiation

It has been well established that the transmitter is released from presynaptic nerve terminals in small packages called quanta in the neuromuscular junction, sympathetic ganglion, terminals of Ia fibers in the spinal cord and other peripheral synapses (Martin 1977). On the other hand, the quantal nature of transmitter liberation has only been studied cursorily in the brain. A finding suggestive of quantal release of the transmitter is the spontaneous fluctuation of membrane potential that has been recorded in vitro from neurons in hippocampal slices under blockade of synaptic transmission with tetrodotoxin (Brown et al. 1979). In the present experiments, I have attempted to estimate quantal content and quantal amplitude of excitatory postsynaptic potentials (EPSPs) elicited in CA3 hippocampal neurons by activation of single mossy fibers (axons of granule cells in the dentate gyrus). This information seems to be important for understanding potentiation of synaptic transmission as well as for analysis of the actions of agents such as synaptic modulators.

In peripheral synapses, the mean number of quanta (m) liberated by an impulse is estimated by dividing the mean amplitude of EPSPs by that of spontaneous miniature EPSPs (Katz 1966). This method cannot be applied to estimate the value of m for mossy fiber terminals, because afferent fibers other than mossy fibers also make synapses on CA3 neurons. It is not certain, therefore, that spontaneously occurring miniature EPSPs represent liberation of quanta of the transmitters solely from mossy fiber terminals. Another routine procedure used in peripheral synapses to estimate the value of m is to divide the number of impulses by the number of failures and then take the logarithm of the quotient (Katz 1966). To apply this method, it is necessary that repeated stimulation always activates all of a group of presynaptic fibers thus excluding that failure of transmission is brought about by an occasional decrease in number of activated fibers. This requirement cannot be satisfied in the case of mossy fibers. To apply a third method (variance method) (Hubbard et al. 1969), therefore, I have attempted to record unitary EPSPs (uEPSPs) which are induced in a CA3 neuron by a single mossy fiber. (The uEPSPs are defined here as EPSPs elicited by individual impulses of single mossy fibers.) On the assumption that the uEPSP amplitudes varied according to

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Poisson's law, the values of \( m \) and quantal amplitude \( (q) \) are calculated from the mean and variance of uEPSP amplitudes.

**Methods**

*Preparation of Tissues and Recording of uEPSPs*

Transverse sections of the hippocampus of the guinea-pig were prepared as described before (Yamamoto 1972) and were incubated in the standard medium at 37\(^\circ\) C for more than 40 min. They were then transferred one by one into an observation vessel which was continuously perfused with the standard medium at 33\(^\circ\) C. A stimulating electrode consisting of a pair of silver wires insulated except for the tips was applied to the granular layer (Fig. 1, S in insert). Intracellular potentials were recorded from neurons in region CA3 with micropipettes filled with 4 M K-acetate. The potentials were amplified both with a low-gain DC and a high-gain AC amplifier, and were displayed simultaneously on an oscilloscope. Although attempts were made to measure the membrane resistance of impaled neurons by passing hyperpolarizing pulses through recording micropipettes, this method was found to be subject to considerable error, because of changes in resistance of micropipettes. Therefore, the values of membrane resistance will not be given. For electrophoretic administration of DL-homocysteate (HCA) to granule cells, double-barrel microelectrodes were used. One barrel of the microelectrode was filled with 0.2 M HCA (pH 8.0) and the other with 3 M NaCl to monitor the firing of granule cells. Diameter of the tip of the double-barrel microelectrode was about 1.5 \( \mu \)m. A retaining current of 20 nA was routinely passed through the HCA barrel except when HCA was ejected. The composition of the standard medium was (mM): NaCl, 124; KCl, 5; KH\(_2\)PO\(_4\), 1.24; MgSO\(_4\), 1.3; CaCl\(_2\), 2.4; NaHCO\(_3\), 26 and glucose, 10. The medium was saturated with 95% O\(_2\) and 5% CO\(_2\).

*Estimation of the Values of m and q*

As shown schematically in Fig. 1 and set forth in the Results, trains of uEPSPs were elicited in a CA3 neuron at an almost constant interval during ejection of HCA at an appropriate site in the granular layer. When judged suitable for statistical treatment according to the criteria specified in the Results, these unitary EPSPs were recorded on running film. When the resting potential deviated more than 3 mV or the noise increased considerably during recording, the position of the intracellular microelectrode was shifted upwards by 1–2 \( \mu \)m. Recording of intracellular potentials was often improved by this procedure. After a sufficient number of uEPSPs were obtained, the electrode was withdrawn and the extracellular potential was recorded. The intracellular records consisted of trains of uEPSPs (Fig. 1b). The amplitude of the first \( (E_1) \) and second uEPSPs \( (E_2) \) in each train were measured. When a second uEPSP occurred partially superimposed, i.e., upon the falling phase of the first, the problem arose as to how to measure the amplitude of this second uEPSP. The procedure adopted was to measure the time for decay to one half maximum amplitude for the falling phase of the first or second uEPSPs (half-decay time, \( t_{1/2} \) in Fig. 1c), and then extended the slope of the interrupted first uEPSP to attain half its amplitude at the half-decay time (broken line in Fig. 1c). The amplitude of the second uEPSP was thus measured from the extrapolated falling phase of the first uEPSP (Fig. 1c). The amplitudes of uEPSPs were corrected for non-linear summation on the assumption that the reversal potential for the EPSP was 0 mV (Martin 1955).

From the above measurements, the value of \( q \) was calculated as the ratio of the variance to the mean of the corrected uEPSP amplitudes. The value of \( m \) was given as the ratio of the mean of the corrected uEPSP amplitudes to \( q \) (Hubbard et al. 1969):

\[
q = \frac{\text{variance of corrected uEPSP amplitudes}}{\text{mean of corrected uEPSP amplitudes}} \quad (1)
\]

and

\[
m = \frac{\text{mean of corrected uEPSP amplitudes}}{q} \quad (2).
\]

**Results**

*Recording of uEPSPs*

Only recordings from neurons with resting potentials of 60–80 mV were evaluated. Spontaneous discharges were not observed in these neurons, and electric stimulation of mossy fibers or short de-