

## Critical Quantum Content for Shortening of Endplate Currents in the Frog Skeletal Muscle

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Received May 6, 1992

Accepted May 20, 1992

### Summary

The decay time of endplate currents was followed during progressive lowering of quantum content of endplate responses by reduced  $\text{Ca}^{2+}$ . A certain critical value of about 100 quanta was found, when the decay of endplate currents remained constant even though the quantal content was reduced further.

### Key words

Motor endplate – Quantal content – Endplate currents

In endplates with inhibited acetylcholinesterase, the prolongation of endplate currents is caused mainly by repetitive interaction of acetylcholine (ACh) with its postsynaptic receptors. This phenomenon is called postsynaptic potentiation (PSP, Hartzell *et al.* 1975, Magazanik *et al.* 1984). Furthermore, in preparations without inhibited cholinesterase, the multiquantal, nerve stimulation evoked EPC decays more slowly than the responses caused by a single quantum (miniature EPC, MEPC) (Glavinovič 1987).

We have studied the time course of responses in relationship to the number of quanta released by nerve stimulation and the decay times of the EPC and MEPC.

Both types of quantal responses were performed on the frog (*Rana ridibunda*) cut sartorius muscles at 20 °C. A standard two-microelectrode voltage clamp recording system was used (Takeuchi and Takeuchi 1959). The muscles were dissected from rapidly decapitated animals and were pinned to the bottom of a translucent chamber. The chamber was superfused with Ringer saline containing (in  $\text{mmol.l}^{-1}$ ): NaCl 115.0, KCl 2.5,  $\text{MgCl}_2$  1.0,  $\text{CaCl}_2$  1.8,  $\text{NaHCO}_3$  2.0, pH = 7.2–7.4. The microelectrodes were inserted into muscle fibres near intramuscular nerve branches and at least 590 MEPCs and 100 nerve stimulus-evoked EPCs (0.2 Hz) were measured in each fibre, filtered at

2.5 kHz, sampled at 60 ms and analyzed by a computer. The quantum content of EPPs was estimated from the ratios of EPC and MEPC amplitudes and changed by lowering of  $\text{CaCl}_2$  in the Ringer solution from 1.8 to 0.1  $\text{mmol.l}^{-1}$ .

The mean MEPC amplitude in 8 endplates was  $1.53 \pm 0.12$  nA (mean  $\pm$  S.E.M), that of EPC was  $316 \pm 17$  nA and the quantum content was  $206 \pm 40$  in the normal Ringer solution. The decays of MEPC and EPC were exponential (from 80 to 20 % of their size) with time constants  $\text{MEPC}_{\text{dec}} = 1.16 \pm 0.06$  ms and  $\text{EPC}_{\text{dec}} = 1.45 \pm 0.06$  ms. The ratio  $\text{EPC}_{\text{dec}}/\text{MEPC}_{\text{dec}}$  was 1.25 showing the longer decay of EPC at normal quantal content of about 200.

To follow the dependence of the ratio  $\text{EPC}_{\text{dec}}/\text{MEPC}_{\text{dec}}$  on the number of quanta, the quantal content was reduced by lowering  $\text{Ca}^{2+}$  in the Ringer solution.

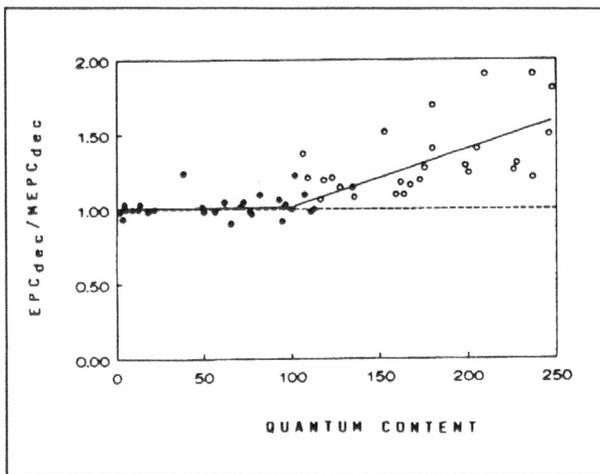
As shown in Fig. 1, the ratio  $\text{EPC}_{\text{dec}}/\text{MEPC}_{\text{dec}}$  approached 1.0 at and below quantal contents of about 100 per nerve impulse due to shortening of the EPC decay. This critical value during lowering of  $\text{Ca}^{2+}$  was found irrespectively of the initial quantal content of each particular endplate which varied from 110 to 250. These data indicate the postsynaptic nature of EPC prolongation. When two or more quanta are released synchronously from one active zone, the increased concentration of ACh near

the receptors may create the opportunity for repetitive binding and prolongation of EPC decay even with intact acetylcholinesterase. The critical quantum content of about 100 might be the number, when the probability that this multiple release at single zones of release is near zero.

Further, more detailed experiments supporting this idea using the decrease of receptor density by competitive blockers which lower the repetitive receptor binding are being submitted for publication elsewhere.

### Acknowledgement

This work was supported by an Internal Grant of the Czechoslovak Academy of Sciences, 1991-2.



**Fig. 1**

Ratio of decay time constants of endplate currents (EPC<sub>dec</sub>) and miniature endplate currents (MEPC<sub>dec</sub>) (ordinate) versus quantum content (abscissa). Each of 56 points represents one set of measurements from one endplate either in normal Krebs solution (open symbols) or 20 min after lowering  $\text{Ca}^{2+}$  from 1.8 to 0.9, 0.4, or 0.1  $\text{mmol.l}^{-1}$  (filled symbols). The regression lines were constructed for both groups of experiments respectively.

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### Reprint Requests

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