Physiol. Res. 54: 129-132, 2005

RAPID COMMUNICATION

Calcium Dependence of Uni-Quantal Release Latencies and Quantal Content at Mouse Neuromuscular Junction

D. SAMIGULLIN[1], E. A. BUKHARAEVA[1,2], F. VYSKOČIL[3], E. E. NIKOLSKY[1,2]

[1]Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, [2]Kazan State Medical University, Kazan, Russia, [3]Department of Animal Physiology and Developmental Biology, Faculty of Sciences, Charles University, Prague, and Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Received September 30, 2004
Accepted November 15, 2004

Summary

Uni-quantal endplate currents (EPC) were recorded at mouse diaphragm neuromuscular synapse by extracellular microelectrode during motor nerve stimulation. The probability of release expressed as quantal content m_o, and variability of synaptic latencies expressed as P_{90} were estimated in the presence of extracellular calcium ($[Ca^{2+}]_o$) varying between 0.2 and 0.6 mM in the bathing solution. At 0.2 mM $[Ca^{2+}]_o$, m_o was low (0.10) and many of long-latency EPCs were present during the late phase of the release (P_{90} = 2.44 ms). No change in m_o was found when $[Ca^{2+}]_o$ was 0.3 mM, but P_{90} decreased by 39 %. For latency shortening, saturating concentration of $[Ca^{2+}]_o$ was 0.4 mM, when P_{90} was 1.49 ms and latencies did not further change at 0.5 and 0.6 mM $[Ca^{2+}]_o$. In the latter concentrations, however, an increase of m_o was still observed. It can be concluded that the early phase of the secretion did not significantly change when $[Ca^{2+}]_o$ was raised and that only the late phase of the release depends on extracellular calcium up to 0.4 mM.

Key words

Quantal release • Synaptic latency • Calcium

In the vertebrate neuromuscular junction, calcium ions play the primary role in promoting the stimulus-induced secretion of acetylcholine (ACh) from nerve terminals (Del Castillo and Katz 1954, Katz 1969, Silinsky 1985, Neher 1998). The efficacy of neurosecretion is determined by two main parameters: the number of transmitter quanta released in response to nerve stimulus (quantal content) and the dispersionof their release (time course of secretion) (Nikolsky *et al.* 2003, Bukharaeva *et al.* 2003). The importance of Ca^{2+} for quantal content is well known (Katz 1969, Llinas 1977, Van der Kloot and Molgó 1994, Zucker 1999, Vyskočil 2004, Augustine 2001 for review), but its role in the time course of secretion is contradictory. In several papers the reciprocal but parallel effect of external Ca^{2+} in the concentrations ranging between 1 and 10 mM on the quantal content m_o and time course of release (latency distribution) was reported (Katz and Miledi 1965, Barrett and Stevens 1972, Minenko and Magazanik 1986, Van der Kloot 1988, Heidelberger *et al.* 1994, Bollmann *et al.*

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

2000, Schneggenburger and Neher 2000). However, the time course of secretion of quanta released either by an action potential or by a direct depolarization was constant under variety of conditions known to change the Ca^{2+} entry (Andreu and Barrett 1980, Datyner and Gage 1980, Parnas *et al*. 1986, Isaacson and Walmsley 1995, Borst and Sakmann 1996). Solving the Ca^{2+} paradox on the quantal content and time course of quanta release could help to decide if there are either common or different molecular mechanisms regulating both processes. In the present paper, we investigated the effect of small extracellular Ca^{+2} concentrations ($[Ca^{2+}]_o$ = 0.2-0.6 mM) on the quantal content and time course of quanta release. In these concentrations, mostly uni-quantal EPCs are recorded in response to nerve stimulation and both processes can be estimated concomitantly.

Isolated diaphragms from the white mice of both sexes (20-25 g body mass) were used. Animals were anesthetized with ether before diaphragm excision. The diaphragm strips were pinned to the bottom of a 1.5 ml translucent chamber and superfused with the following low-Ca^{2+}, high-Mg^{2+} solution (mM): NaCl 120.0, KCl 5.0, $CaCl_2$ 0.2-0.6, $NaHCO_3$ 11.0, NaH_2PO_4 1.0, $MgCl_2$ 4.0, glucose 11, pH 7.3-7.4. The experiments were performed at 20.0±0.3 °C. Suprathreshold 0.1 ms rectangular stimuli generated by WPI A385R High Current Stimulus Isolator were applied to the phrenic nerve at 2 s intervals *via* a suction electrode filled with an extracellular solution. Extracellular nerve spikes and endplate currents were recorded using a heat polished electrode with 2-4 μm tip diameter filled with extracellular solution. Electrode was positioned near nerve ending in the site where triphasic nerve action potential (NAP) can be recorded (Mallart and Brigant 1982). Signals were filtered and processed by our application program. The quantal contents (m_o) of the low-quanta EPC were determined by measuring the EPCs during five or six stimulation periods (256 stimuli each) by the failure method. The numbers of failures were measured and m_o was calculated as equal to [ln N/n_o], where N is the total number of stimuli and n_o is the number of failures (Del Castillo and Katz 1954, Martin 1955). To estimate the time course of transmitter quanta release, only uni-quantal endplate currents are required (Katz and Miledi 1965, Barrett and Stevens 1972). Therefore, in each experiment in low-Ca^{2+}, high-Mg^{2+} solution, histograms of EPC amplitudes were analyzed and only signals corresponding to the first histogram peak (i.e. real uni-quantal EPCs, cf. Boyd and Martin 1956) were used for latency measurements (not shown, cf. Bukcharaeva *et al*. 1999). Latency was estimated as the time interval between the peak of the inward part of presynaptic current and the time at which the rising phase of the quantal event reached 20 % of maximum (Fig.1, left panels, red arrows). The mean value of the shortest 5 % of latencies in each series was taken as the minimal synaptic delay (Barrett and Stevens 1972). Statistical analyses were performed using Student's *t*-test for paired data. The quantitative characteristics of the time course of evoked secretion was obtained by cumulative curve analysis (Van der Kloot 1991, Bukcharaeva *et al.* 1999). The cumulative curves were constructed from latency histograms of the uni-quantal EPCs (for details see Bukcharaeva *et al.* 1999). For estimation of latency dispersion, parameter P_{90} represents the interval between the minimal synaptic delay and the time at which 90 % of all measured uni-quantal EPCs had occurred (Samigullin *et al*. 2003).

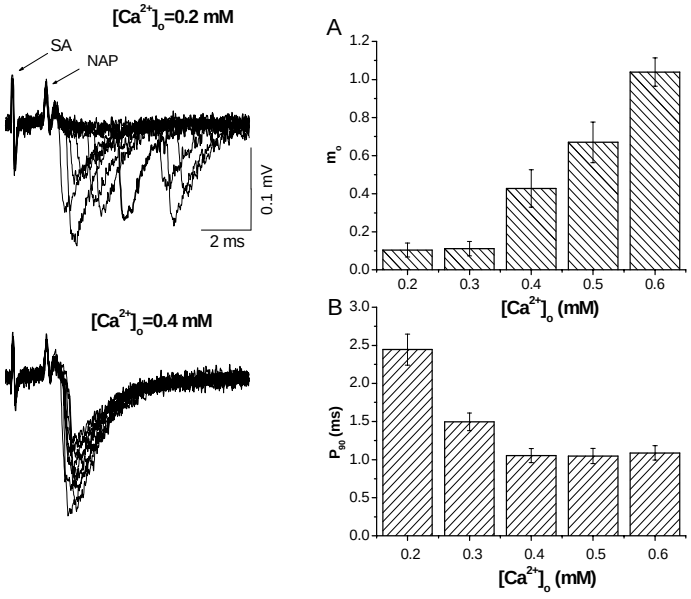

Fig. 1. The effect of extracellular calcium on quantal content and release latencies. Left panels – 9-10 traces were superimposed, showing extracellularly recorded stimulus artifacts (SA), presynaptic nerve action potentials (NAP) and individual endplate currents. The time interval between the downward peak of the presynaptic NAP and the times at which the rising phases of each EPC reached 20 % of maximum was defined as the release latency. Note the pronounced decrease in latency dispersion at higher concentration (0.4 mM) of the extracellular concentrations of calcium ($[Ca^{2+}]_o$). A – quantal content m_o (ordinate) and B – quantal release latencies (expressed as P_{90}) in the presence of different $[Ca^{2+}]_o$, (abscissa in A and B). Mean values ± S.E.M. from 7-10 endplates are shown.

In most experiments, both parameters m_o and P_{90} were measured simultaneously on single endplate which was superfused progressively by solutions with six gradually rising concentrations of $[Ca^{2+}]_o$. At 0.2 mM $[Ca^{2+}]_o$, m_o was low (0.10±0.03) and a number of long-latency EPCs were present during the late phase of the release (P_{90} = 2.44±0.20 ms). No change in quantal content m_o was found when $[Ca^{2+}]_o$ was increased to 0.3 mM (Fig.1 A), whereas P_{90} significantly decreased by 39 %, from 2.44±0.20 ms to 1.49±0.11 ms ($P<0.05$, n=10) (Fig. 1B). Further rise of $[Ca^{2+}]_o$ up to 0.6 mM led to a gradual increase of m_o, which was 0.43±0.06 at 0.4 mM $[Ca^{2+}]_o$, 0.67±0.11 at 0.5 mM $[Ca^{2+}]_o$ and 1.03±0.07 ($P<0.05$, n=10) at 0.6 mM $[Ca^{2+}]_o$. On the contrary, latency shortening expressed as decrease of P_{90} stopped to decline at 0.4 mM $[Ca^{2+}]_o$ when it reached the shortest values of about 1.0 ms which was maintained in 0.5 and 0.6 mM $[Ca^{2+}]_o$. The saturating concentration of $[Ca^{2+}]_o$ for latency dispersion (P_{90}) was therefore 0.4 mM. Noteworthy, early phase of the secretion characterized by minimal latencies and main mode of the latency histograms did not significantly change when $[Ca^{2+}]_o$ was elevated from 0.2 to 0.6 mM $[Ca^{2+}]_o$. Minimal latencies varied between 0.42 ms to 0.48 ms in all concentrations of $[Ca^{2+}]_o$ and main mode of the latency histograms were in the range of 0.63 ms and 0.66 ms. Therefore, only the late phase of the release, when latencies are longer than 1 ms (Barrett and Stevens 1972) depends on extracellular calcium in such concentration range, where direct latency measurements of individual uni-quantal EPCs are still possible.

Changes in the time course of entry and removal of calcium are considered to influence both the number of quanta (m_o) and the kinetics of quantal release. However, there are data showing that the time course of the release remains the same even with several fold change in the quantal content (Andreu and Barrett 1980). On the other hand, we demonstrated that quantal content remained unaltered while the time course of the release became shorter in the presence of noradrenaline, other β_1 agonists and during elevation of the intracellular cAMP (by application of permeable cAMP derivative, inhibition of cAMP degradation and by activation its production) (Bukharaeva *et al*. 1999, 2002, Vyskočil 2004). It indicates that more than one presynaptic mechanism can control synaptic quantal neurosecretion efficacy (Parnas and Parnas 1999). Moreover, present results demonstrate that both release parameters can be altered independently by one simple maneuver – by fine setting of extracellular Ca^{2+}: m_o is constant and P_{90} decreases between 0.2 and 0.3 mM $[Ca^{2+}]_o$ and m_o rises and P_{90} is constant between 0.4 and 0.6 mM $[Ca^{2+}]_o$. It also follows that the mechanism controlling of the time course of release saturates more quickly than that regulating the quantal content. Taken together, the present data suggest that different mechanisms are involved in the control of quantal content and release kinetics even if triggered by one stimulus, i.e. Ca^{2+} ions.

Acknowledgments

Supported by GA AS CR A5011411, GA CR 305/02/1333 and MSMT 11310003 for FV, RFBR 05-04-49723 and Scientific Schools of Russia 1063.2003.4 for EN, by "Russian Science Support Foundation" and MK-2153.2003.04 for DS.

References

ANDREU R, BARRETT E: Calcium dependence of evoked transmitter release at very low quantal content at the frog neuromuscular junction. *J Physiol Lond* **308**: 79-97, 1980.

AUGUSTINE GJ: How does calcium trigger neurotransmitter release. *Curr Opin Neurobiol* **11**: 320-326, 2001.

BARRETT E, STEVENS C: The kinetics of transmitter release at the frog neuromuscular junction. *J Physiol Lond* **227**: 691-708, 1972.

BOLLMANN JH, SAKMANN B, BORST JG: Calcium sensitivity of glutamate release in a calyx-type terminal. *Science* **289**: 953-957, 2000.

BORST JG, SAKMANN B: Calcium influx and transmitter release in a fast CNS synapse. *Nature* **383**: 431-434, 1996.

BOYD I, MARTIN A: Spontaneous subthreshold activity at mammalian neuromuscular junction. *J Physiol Lond* **132**: 61-73, 1956.

BUKCHARAEVA E, KIM K, MORAVEC J, NIKOLSKY E, VYSKOČIL F: Noradrenaline synchronizes evoked quantal release at frog neuromuscular junctions. *J Physiol Lond* **517**: 879-888, 1999.

BUKHARAEVA E, SAMIGULLIN D, NIKOLSKY E, VYSKOČIL F: Protein kinase A cascade regulates quantal release dispersion at frog muscle endplate. *J Physiol Lond* **538**: 837-848, 2002.

BUKHARAEVA E, SAMIGULLIN D, NIKOLSKY E, ADÁMEK S, VYSKOČIL F: Quantal latencies and protein kinases at frog muscle endplate. *Physiol Res* **52**: 25P, 2003.

DATYNER N, GAGE P: Phasic secretion of acetylcholine at a mammalian neuromuscular junction. *J Physiol Lond* **303**: 299-314, 1980.

DEL CASTILLO J, KATZ B: Statistical factors involved in neuromuscular facilitation and depression. *J Physiol Lond* **124**: 574-585, 1954.

HEIDELBERGER R, HELNEMANN C, NEHER E, MATTHEWS G: Calcium dependence of the rate of exocytosis in a synaptic terminal. *Nature* **371**: 513-515, 1994.

ISAACSON J, WALMSLEY B: Counting quanta: direct measurements of transmitter release at a central synapse. *Neuron* **15**: 875-884, 1999.

KATZ B: The release of neural transmitter substances. *The Sherrington Lectures X.* Liverpool University Press, Liverpool, UK, 1969.

KATZ B, MILEDI R: The measurement of synaptic delay, and the time course of acetylcholine release at the neuromuscular junction. *Proc R Soc Lond B Biol Sci* **161**: 483-495, 1965.

LLINÁS R: Calcium and transmitter release in squid synapse. *Soc Neurosci Symp* **2**: 139-160, 1977.

MALLART A, BRIGANT J-L: Electrical activity at motor nerve terminals of the mouse. *J Physiol Paris* **78**: 407-411, 1982.

MARTIN AR: A further study of the statistical composition of the endplate potential. *J Physiol Lond* **130**: 114-122, 1955.

MINENKO ML, MAGAZANIK LG: Phenomena of asynchronous evoked transmitter release at the neuromuscular junction of the frog. *Neurophysiology* **18**: 346-354, 1986.

NEHER E: Vesicle pools and Ca^{2+} microdomains: new tools for understanding their roles in neurotransmitter release. *Neuron* **20**: 389-399, 1998.

NIKOLSKY E, SAMIGULLIN D, BUKHARAEVA E, ADÁMEK S, VYSKOČIL F: Proximal to distal gradient of temperature sensitivity of quantal acetylcholine release at frog muscle endplate. *Physiol Res* **52**: 35P, 2003.

PARNAS H, DUDEL J, PARNAS, I: Neurotransmitter release and its facilitation in crayfish VII. Another voltage-dependent process besides Ca^{2+}-entry controls time course of phasic release. *Pflügers Arch.* **406**: 121-130, 1986.

PARNAS I, PARNAS H: Different mechanisms control the amount and time course of neurotransmitter release. *J Physiol Lond* **517**: 629, 1999.

SAMIGULLIN D, BUKHARAEVA E, NIKOLSKY E, ADÁMEK S, VYSKOČIL F: Long release latencies are increased by acetylcholine at frog endplate. *Physiol Res* **52**: 475-480, 2003.

SCHNEGGENBURGER R, NEHER E: Intracellular calcium dependence of transmitter release rates at a fast central synapse. *Nature* **406**: 889-893, 2000.

SILINSKY E: The biophysical pharmacology of calcium-dependent acetylcholine secretion. *Pharmacol Rev* **37**: 81-132, 1985.

VAN DER KLOOT W: The kinetics of quantal releases during end-plate currents at the frog neuromuscular junction. *J Physiol Lond* **402**: 605-626, 1988.

VAN DER KLOOT W: The regulation of quantal size. *Progr Neurobiol* **36**: 93-130, 1991.

VAN DER KLOOT W, MOLGÓ J: Quantal acetylcholine release at the vertebrate neuromuscular junction. *Physiol Rev* **74**: 899-991, 1994.

VYSKOČIL F: Principles of adrenergic and cholinergic regulation of quanta release dispersion at frog endplate. *Physiol Res* **53**: 44P, 2004.

ZUCKER RS: Calcium- and activity-dependent synaptic plasticity. *Curr Opin Neurobiol* **9**: 305-313, 1999.

Reprint requests

Dr. František Vyskočil, Institute of Physiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic. E-mail: vyskocil@biomed.cas.cz