Miniature Excitatory Synaptic Ion Currents in the Earthworm *Lumbriterrestris* Body Wall Muscles

E.M. VOLKOV[1], L.F. NURULLIN[2], E. NIKOLSKY[1,2] AND F. VYSKOČIL[3,4,*]

[1]*State Medical University, Kazan, Russia*

[2]*Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russia*

[3]*Department of Animal Physiology and Developmental Biology, Charles University, Prague, Czech Republic and*

[4]*Institute of Physiology, Czech Academy of Sciences, Prague*

[*] Author for correspondence, vyskocil@biomed.cas.cz

Key words

Acetylcholine receptor • Ion currents • Muscle cells • Earthworm

Summary

The miniature excitatory postsynaptic currents (MEPCs) of the muscle cells of the earthworm *Lumbricus terrestris* were recorded by glass microelectrodes. In a single synaptic zone, three types of MEPC were recorded: a fast single-exponential type that decayed with τ =0.9 ms, a slow single-exponential with τ = 9.2 ms and a two-exponential MEPC with τ = 1.3 and 8.5 ms, respectively. The muscle cells of earthworms contain populations of yet-unidentified ionic channels that might be different from the common nicotinic and muscarinic groups of acetylcholine receptors, since these MEPCs are not sensitive to d-tubocurarine, atropine, benzohexonium or proserine. Alternatively, besides ACh receptors, the membrane may contain receptors for another yet-unidentified excitatory transmitter.

It is generally accepted that the transmission of excitation from the motor nerves to the body muscles of the earthworm is cholinergic and quantal in nature (Rozkova 1973, Walker et al. 1993). While studying the electrophysiological properties of the postsynaptic muscle membrane

(Volkov et al. 2000, 2003) using a microelectrode current clamp method, we have found that small spontaneous voltage excursions, miniature endplate potentials (MEPPs), are very variable in their time course. There are two possible explanations for such variability, which is much more pronounced in earthworms than in any vertebrate neuromuscular synapses (Magazanik et al. 1979); MEPPs are either distorted by the cable properties (high membrane capacitance) of the postsynaptic muscle fibre or there are different populations of transmitter quanta acting on receptor/channel complexes with different open times (Jaksta-Sauerland and Coggeshall 1973).

To distinguish between these two possibilities, miniature spontaneous events were recorded extracellulary by a glass micropipette as local ionic currents. This approach (Bukharaeva et al. 2005) to a large extent eliminates the membrane capacitance and cable properties of the postsynaptic muscle fibre that distort the amplitude and time course of the voltage-recorded miniature signals (Samigullin et al. 2005). It also enables the determination of whether the sources of the long and short signals are nearby (within 1-2 μm) or originate from more distant locations.

Methods

Experiments were performed on isolated neuromuscular preparations of the longitudinal somatic muscles of the earthworm *Lumbricus terrestris* (Drewes and Pax 1974). Strips of the earthworm body wall muscle were prepared, approximately 10 centimetres in length, from which the nerve cord and viscera had been removed and placed in earthworm-modified Drewes-Pax solution (Drewes and Pax 1974), which contained (mM): NaCl, 163; KCl, 4; $CaCl_2$, 6; sucrose, 167; Tris, 2; pH 7.2 - 7.4. Endplate currents were recorded extracellularly with glass microelectrodes (filled with 2.5 mol/l NaCl, 3-5 MΩ) carefully placed on the muscle fibre membrane near the motor nerve endings, verified by stereoscopic microscope Meade Model 8300 Stereo, Northbrook, IL. U.S.A. (40-100x). In most cases, 150-200 EPCs events were captured from each fibre in the control and 15-20 min after application of the particular drug, and analyzed by a computer for frequency, amplitudes, rise times (from 10 to 90 % of the maximal amplitude) and exponential decay constants τ_{dec}. In each experimental group, 4-6 animals were used.

Microcal Origin, version 3.5, (Microcal Software, Inc. 1991-1994) was used for statistical analyses. A parametric analysis of variance (ANOVA) of the experimental groups *versus* the control group was made by multiple comparison using the Bonferroni t-test. Throughout the text,

statistically significant differences between the mean ± S.E.M. of the two groups are indicated at the given level of probability P.

Results and Discussion

Three types of MEPCs were recorded in virtually all synaptic zones of the body wall muscle fibres. The first type were “fast” signals having a rise time of 0.50±0.06 ms (n=300, 8 muscles) and a single exponential decay phase with a τ which varied from 0.5 to 1.2 ms (0.9±0.07 ms, n=300, 8 muscles) (Fig. 1). Their mean amplitude was 0.27±0.04 mV (n=300, 8 muscles). This type of MEPC accounted for 34±3% of all events recorded from total 67 fibres.

Second type was a “slow” one with a similar mean but more dispersed range of amplitudes (0.3±0.2 mV , n=300, 8 muscles) having a rise time of 0.6±0.03 ms (n=300, 8 muscles) and also only a single exponential decay phase. This decay was, however, very prolonged and the τ of the MEPC decrease was almost ten times greater than in “fast” MEPCs ranging from 8 to 10 ms (τ=9.2±0.09 ms, n=300, 8 muscles). This “slow” type of MEPC accounted for 34±4% of all events recorded from the total of 67 fibres.

There was also a third “mixed” type, with a relatively uniform rise time of about 0,6 ms, but with two distinct values of the exponential amplitude decrease, a τ_1 of approximately 1,3 ms corresponding closely to the fast type and a τ_2 of about 8,5 ms, corresponding to the “slow” type. The third type is mostly hidden and can be revealed only after statistics of parameters is performed. It can hardly be visible on the native recordings such as Fig. 1.

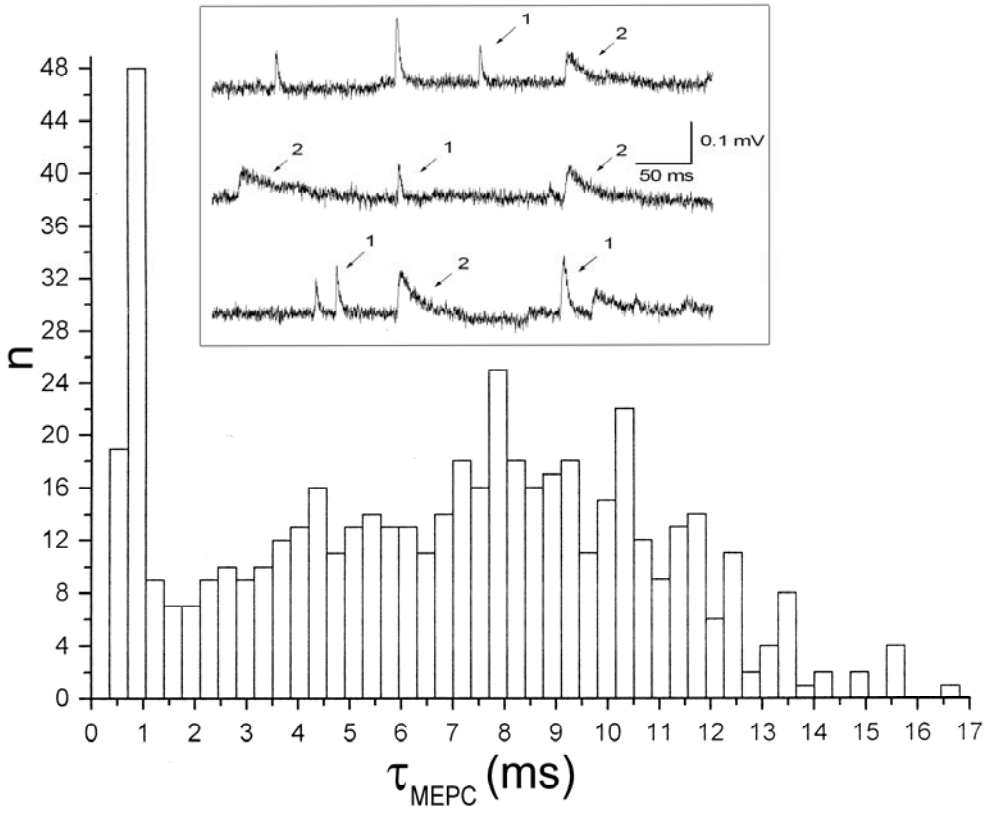

Fig. 1. Histogram of distribution of MEPC exponential decay constants (in ms) in one endplate. n = number of MEPCs, inset = records of fast and slow MEPCs, indicated by 1 or 2 respectively.

All three types of signal are usually recorded by a single focal extracellular electrode in one synaptic zone. We can speculate that very close each other, probably at a single endplate zone, there are either 1) two kinds of quanta, each filled with different transmitter, similarly to ACh and serotonin in leech polyneural endplates (Jaksta-Sauerland and Coggeshall 1973, David 1990) which can open the same type of channel for short and long periods, 2) there are two distinct areas with two kinds of quanta, each filled with a different isotransmitter, 3) vesicles are filled with identical transmitters, but channels can be opened by the same transmitter for either short or long periods.

The first possibility is difficult to test, but experiments with different cholinergic antagonists (see below) suggest that it may not be the case. The second possibility was

demonstrated experimentally by artificially filling vesicles with ACh derivatives, which led to a shortening or prolongation of MEPCs (e.g. ACh and monoethylcholine, Colquhoun et al. 1977) However, to our knowledge, no such subpopulations of “isotransmitters” were reported in native endplates, either invertebrate or vertebrate.

On the other hand, two subtypes of receptors are a more plausible explanation for the different time courses- ACh nicotinic receptors (AChRs) with different properties were discovered at vertebrate endplates a long time ago. There are adult nAChRs with an MEPC τ of about 1.2 ms and mean open channel conductance of approximately 60 pS. Much slower fetal or denervation nAChRs (Beránek and Vyskočil, 1967) have a τ of 7-8 ms and conductance of about 40 pS (Michler and Sakmann 1980, Witzemann et al. 1987 FEBs). These two types of receptors are hardly ever present at the same time in the same vertebrate endplate in comparable amounts and there is a continuous switching of the nAChR expression program from predominantly the fetal (until postnatal day 7 in rat) to predominantly the adult type (from postnatal day 21 in rat, Vicini and Schuetze 1985). The simultaneous presence of both types of MEPCs in earthworm more likely demonstrates that there are quanta that open receptor zones with predominantly long or short-openings and that some of these quanta can accidentally overlap both zones giving rise to MEPCs with the intermediate decay time. The overall conductance of both receptor zones is very similar, and no significant correlation was found between the MEPC's amplitude and decay time (coefficient of correlation $R = 0.39$, $P < 0.0001$).

Despite the sensitivity of the postsynaptic muscle fibres to bath-applied acetylcholine and carbacholine (that can depolarize the earthworm muscle and cause muscle contraction) and the presence of synaptic acetylcholine esterase, neither the nature of released transmitters nor the type of receptors were yet determined for these spontaneous MEPCs. In our experience (data in preparation), the amplitude and decay time of both the short and long types of MEPCs are insensitive to the nicotinic antagonist d-tubocurarine, muscarinic antagonist atropine, ganglionic antagonist benzohexonium (Brown 1980; Rang 1982) and anticholinesterase proserine in concentrations up to 10^{-4} M. In addition, other classic adrenergic, serotoninergic and glutamatergic drugs were ineffective and further studies on the pharmacologic characterization of both types of earthworm MEPCs are underway. In particular, the attention will be paid to peptidergic systems such as FMRamide (Csoknya et al. 2005). It is evident that efferent system of

the annelid body wall may operate by means of a variety of neuroactive compounds, suggesting a complex role of signalling systems in the regulation of this neuro-muscular system

References

BERANEK R, VYSKOCIL F: The action of tubocurarine and atropine on the normal and denervated rat diaphragm. *J Physiol* **188(1)**: 53-66, 1967.

BROWN DA: Locus and mechanism of action of ganglion-blocking agents. *In Pharmacology of Ganglionic Transmission, ed. Kharkevich, D.A. Berlin: Springer*: 185-235, 1980.

BUKHARAEVA EA, SALAKHUTDINOV RI, VYSKOCIL F, NIKOLSKY EE: Spontaneous quantal and non-quantal release of acetylcholine at mouse endplate during onset of hypoxia. *Physiol Res* **54(2)**: 251-255, 2005.

COLQUHOUN D, LARGE WA, RANG HP: An analysis of the action of a false transmitter at the neuromuscular junction. *J Physiol* **266(2)**: 361-395, 1977.

CSOKNYA M, TAKACS B, KOZA A, DENES V, WILHELM M, HIRIPI L, KASLIN J, ELEKES K: Neurochemical characterization of nervous elements innervating the body wall of earthworms (Lumbricus, Eisenia): immunohistochemical and pharmacological studies. *Cell Tissue Res 321***(3)**:479-490, 2005.

DAVID OF: Morphophysiological basics of locomotion of annelids. *L. Nauka*, 1990.

DREWES CP, PAX RA: Neuromuscular physiology of the longitudinal muscle of the earthworm, Lumbricus terrestris. I. Effect of different physiological salines. *J Exp Biol* **60**: 445-452, 1974.

JAKSTA-SAUERLAND BA, COGGESHALL RE: Neuromuscular junction in the leech. *J Comp Neurol* **151**: 85-99, 1973.

MAGAZANIK LG, FEDOROV VV, SNETKOV VA: The time course of postsynaptic currents in fast and slow junctions and its alteration by cholinesterase inhibition. *Prog Brain Res* **49**: 225-240, 1979.

MAGLEBY KL, STEVENS CF: The effect of voltage on the time course of end-plate currents. *J Physiol* **233(1)**: 173-197, 1972.

MICHLER A, SAKMANN B: Receptor stability and channel conversion in the subsynaptic membrane of the developing mammalian neuromuscular junction. *Dev Biol* **80(1)**: 1-17, 1980.

RANG HP: Drugs and ionic channels: mechanisms and implications. *Postgrad Med J* **57(Suppl 1)**: 89-97, 1981.

ROZKOVA EK: Pharmacology of cholinergic systems in annelids. *International encyclopedia of pharmacology and therapeutics. Sect.85 Oxford; New York* **1**: 169-189, 1973.

SAMIGULLIN D, BUKHARAEVA EA, VYSKOCIL F, NIKOLSKY EE: Calcium dependence of uni-quantal release latencies and quantal content at mouse neuromuscular junction. *Physiol Res* **54(1)**: 129-132, 2005.

VICINI S, SCHUETZE SM: Gating properties of acetylcholine receptors at developing rat endplates. *J Neurosci* **5(8)**: 2212-2224, 1985.

VOLKOV EM, NURULLIN LF, SVANDOVA I, NIKOLSKY EE, VYSKOCIL F: Participation of electrogenic Na^+-K^+-ATPase in the membrane potential of earthworm body wall muscles. *Physiol Res* **49(4)**: 481-484, 2000.

VOLKOV EM, NURULLIN LF, NIKOLSKY E, KRUSEK J, VYSKOCIL F: Chloride cotransport in the membrane of earthworm body wall muscles. *Physiol Res* **52(5)**: 587-592, 2003.

WALKER RJ, HOLDEN-DAY L, PRANKS CJ: Physiological and pharmacological studies on annelid and nematode body wall muscle. *Comp Biochem Physiol* **106(1)**: 49-58, 1993.

WITZEMANN V, BARG B, NISHIKAWA Y, SAKMANN B, NUMA S: Differential regulation of muscle acetylcholine receptor gamma- and epsilon-subunit mRNAs. *FEBS Lett* **223(1)**: 104-112, 1987.