Two Populations of Miniature Excitatory Synaptic Ionic Currents in Somatic Muscle Cells of *Lumbricus Terrestris* Earthworm Body Wall

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Three types of miniature excitatory synaptic currents were recorded in the same synaptic region of earthworm muscle cells: monoexponential (τ=1.2 msec) and biexponential (τ_1=1.2 and τ_2=8.0 msec). It was hypothesized that earthworm muscle cells contain at least two populations of acetylcholine-sensitive ionic channels, which do not belong to classical nicotinic and muscarinic acetylcholine receptors.

Key Words: *acetylcholine receptor; ionic currents; muscle cells; earthworm*

Excitation transmission from motor nerves to somatic muscles is considered to have a cholinergic nature [6]. However, according to pharmacological criteria, the postsynaptic receptor-channel complex of the muscle cells, which is sensitive to cholinomimetics, cannot be related to any classical type of acetylcholine (Ach) complexes [4,8]. Our aim was to analyze properties of postsynaptic excitatory signals in order to elucidate the nature of postsynaptic chemosensitive ionic channels of somatic muscle cells in earthworm.

MATERIALS AND METHODS

Experiments were carried out on isolated muscle cells from longitudinal muscle bundles of internal coelomic wall of *Lumbricus terrestris* earthworm. Freshly isolated coelomic fragments (10-15 segments) were cut longitudinally, cleaned from coelomic organs, and placed in modified Dreves-Pax solution [3,7] containing (in mM): 163 Na^+, 4 K^+, 6 Ca^{2+}, 93 Cl^-, 43 SO_4^{2-}, 2 $Tris^+$, 167 sucrose. Osmolarity, ionic strength, and pH at room temperature were 478 mosmol/liter, 229 mM and 7.2-7.4, respectively.

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Miniature excitatory postsynaptic currents (MEPSC) of muscle cells were recorded with glass microelectrodes filled with 0.5 mM NaCl (tip resistance ~1 MΩ). Real-time analysis of the amplitude-time characteristics of the miniature excitatory postsynaptic potentials (MEPSP) and MEPSC was performed with a digitizer and a computer (Intel-Pentium 3, 1.2 GHz). In each experiment on a single muscle cell, the mean potential amplitude (mV), rise time (msec), and decay time (msec) of MEPSP and MEPSC were obtained from averaging no less than 1000 discrete points [5,7]. The correlation between the decay time of monoexponential miniature currents and their amplitude was calculated with original software. To this end, the signals were subdivided into monoexponential and biexponential synaptic currents.

RESULTS

Analysis of extracellular MEPSC showed that there are three types of these currents in the synaptic area of muscle cells. The first type of MEPSC is represented by monoexponential fast signals with rise time of 0.50±0.06 msec and the decay time of 0.5-1.2 msec (Fig. 1). MEPSC of the second type are monoexponential slow signals with rise time of 0.50±0.06 msec and decay time of 8-10 msec (Fig. 1). MEPSC of the third

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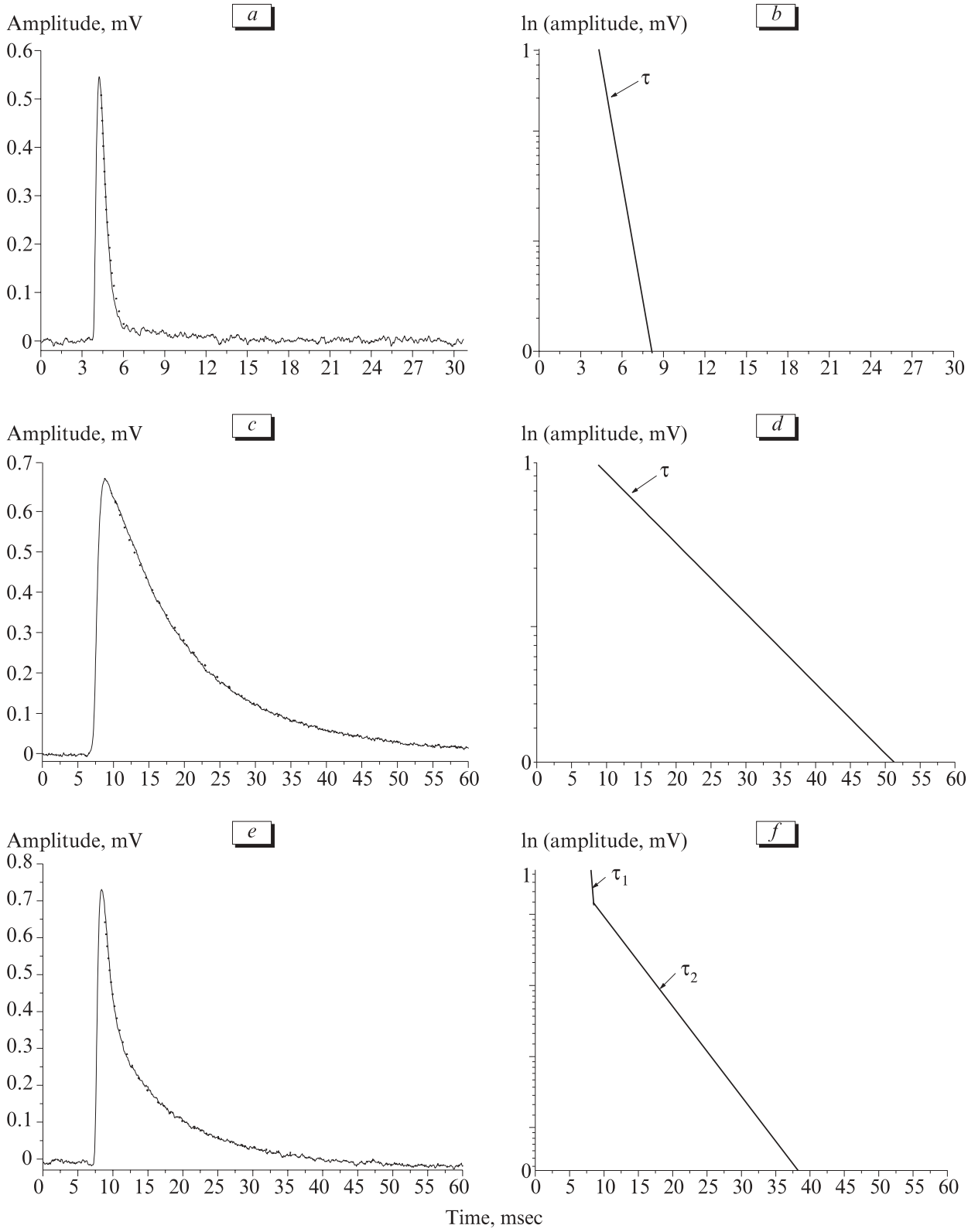

Fig. 1. Extracellular miniature excitatory postsynaptic currents (MEPSC, the mean values) of somatic muscle cells from earthworm coelom. Shown are the fast (*a*, *b*), slow (*c*, *d*), and mixed (*e*, *f*) currents. *a, c, e*) linear scale; *b, d, f*) natural logarithmic scale. Dashed line is monoexponential approximation of falling edge for the fast and slow currents and biexponential approximation for mixed currents. The arrows indicate regions where decay time constants of each exponential curve were determined.

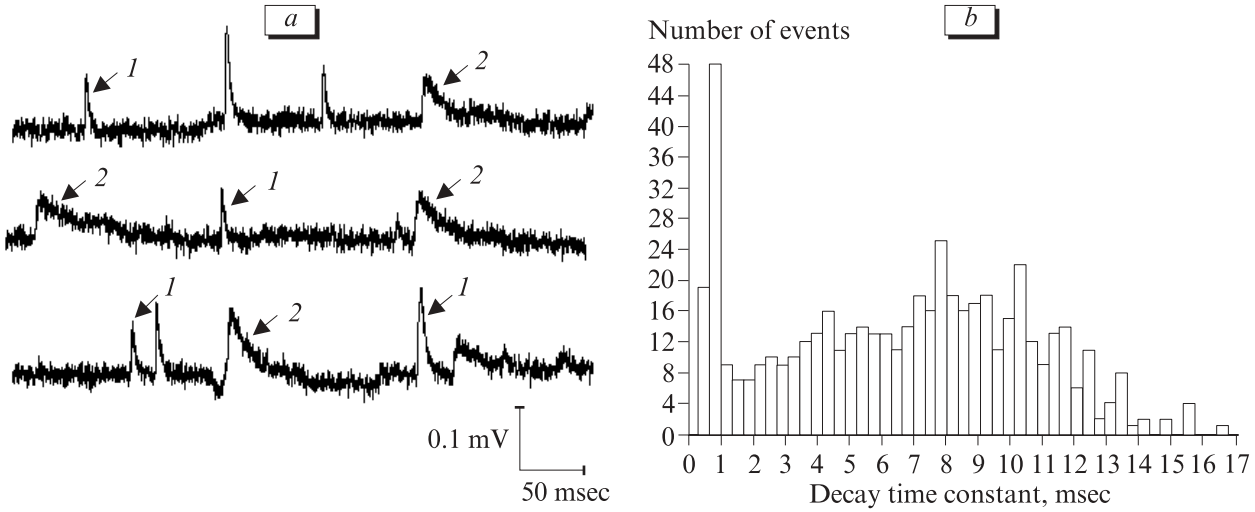

Fig. 2. MEPSC and histogram of MEPSC decay time constant t in individual earthworm muscle cell. *a*) fast (*1*) and slow (*2*) MEPSC; *b*) histogram of MEPSC decay time constants.

type are mixed signals with biexponential decay. They consisted of two processes with rise and decay times 0.6 and 1.3 msec for the first process and 0.6 and 8.5 msec for the second process (Fig. 1). The signals of all types were recorded in the same synaptic area (Fig. 1 and 2, *a*), which is attested by polymodal distribution of MEPSC decay times (Fig. 2, b), so the differences in the decay times cannot be explained by individual deviations of resting potential in various muscle cells [7]. It is also confirmed by the absence of correlation (R=0.39) between the amplitude and decay time of MEPSC. Thus, at least two types of channel-receptor complexes are available in the area of a single motor synapse, which differ in open state duration of the ionic pore [5,7]: the slow complexes with the open state duration of about 8 msec and the fast ones with the decay time of 1 msec. The fast complexes are similar to Ach-channel complexes of the higher innervated vertebrate muscles in the matter of the open state duration, while open state duration of the slow complexes is almost 2-fold larger than similar value even in Ach-channel complexes of denervated muscles [2]. Recordings of MEPSC of different types (fast, slow, mixed) attests to existence of individual sites in the same synaptic area, which differ in the density of ionic channels with small and large duration of the open state. The existence of mixed (biexponential) currents indicates the existence of overlapping sites, in which a significant number of both types of receptor-channel complexes are available. There can be a variety of reasons explaining this heterogeneity of chemosensitive ionic channels in the postsynaptic membrane of earthworm muscle cells. This heterogeneity most likely results from structural and morphological peculiarities of this animal. Earthworms are characterized by polyneural multiterminal innervation of somatic musculature [6]. In Annelida leeches, the polyneural innervation of the same synaptic region was established, where two different morphological types of presynaptic nerve terminals are available, which presumably release various excitatory transmitters such as Ach and serotonin [6]. It could be hypothesized that different types of MEPSC result from interaction of various excitatory mediators with the corresponding postsynaptic receptors available in a single muscle cell, where slow currents are triggered by one excitatory transmitter, while fast currents are induced by another transmitter. However, this hypothesis seems to be improbable, because it suggests a synchronous release of different transmitters from virtually the same morphological structure. There is a more realistic approach. It is well known that in vertebrate denervated muscles, the "normal" receptors characteristic of innervated muscle (open time 1.2 msec) are replaced by "abnormal" receptors with open time of 4 msec [1,2]. These changes are explained by the fact that loss of neurotrophic control results in reprogramming of expression of a family of genes, which are responsible for the synthesis of protein subunits forming Ach-sensitive receptor-channel complex in myofibrils [1]. It could be suggested that muscle cells of earthworm simultaneously synthesize several types of protein subunits of Ach-receptors that determine open time of the ionic pore. Under the effect of neurotrophic control and other local factors, these subunits aggregate to form the receptor-channel complexes with various open time ("fast" and "slow" complexes). This heterogeneity of population of Ach-receptors probably results from the following observation: although the neurotrophic control responsible for different synthesis of Ach-receptors in innervated and denervated muscles in vertebrates [1] works also in earthworm

[6], it has no unambiguous effect on the genome of muscle cells in this animal.

Taking into consideration that nicotinic and muscarinic antagonists and ganglioblockers do not remove depolarization of muscle membrane induced by Ach or carbacholine [4], it can be hypothesized that muscle cell membrane in earthworm contain the chemosensitive ionic channels excited by Ach. These receptor-channel complexes have at least two populations of Ach-receptors with different open times of the ionic pore. The open time of one population is similar to that of nicotinic receptor-channel complex of innervated skeletal muscles of higher vertebrates [2], while open time of other population is almost one order of magnitude longer and has no corresponding analog. Both fractions of chemosensitive ionic channels cannot be attributed to any known pharmacological types of Ach-receptors in muscles and peripheral neurons of the vertebrates.

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