RESTING MEMBRANE POTENTIALS AND MINIATURE POSTSYNAPTIC POTENTIALS OF FROG INTRAFUSAL MUSCLE FIBERS

E. A. Yurkyanets

UDC 612.743

By recording intracellular potentials the electrophysiological characteristics of the intrafusal muscle fibers of the frog muscle spindle were investigated. Analysis of the distribution of amplitudes of the miniature postsynaptic potentials indicates heterogeneity of the intrafusal muscle fibers. It is concluded that the frog muscle spindle contains three types of muscle fibers: those with single innervation, and those combining the two types of innervation.

KEY WORDS: *extrafusal fiber; intrafusal fiber; resting membrane potential; resting miniature potential.*

In amphibians the muscle spindles are known to have an intrinsic motor apparatus consisting of a bundle of between 3 and 12 thin intrafusal muscle fibers. Histochemical methods of investigation have shown the heterogeneity of the fiber composition of the intrafusal muscle bundle in frogs [6]. Two types of end plates (twitch and grape), characteristic of fast (jerk, phasic) and slow (tonic) fibers respectively, have been identified in the intrafusal fibers. With the aid of intracellular microelectrodes action potentials have been recorded in some intrafusal fibers [5], and this has been interpreted as evidence of the presence of the fast muscle fibers in the intrafusal bundle. Only indirect evidence of the presence of slow muscle fibers in the spindles has so far been obtained in the form of physiological data showing an increase in the afferent discharge frequency of the spindle during stimulation of efferent nerve fibers of small diameter, as a rule innervating slow muscle fibers, not conducting excitation [1, 5]. However, several workers have shown that the same intrafusal fibers can be innervated and activated by efferent fibers of both large and small diameter [4, 6]. Such muscle fibers probably combine both types of function (phasic and tonic).

The object of this investigation was to study fibers of the intrafusal muscle bundle of the frog muscle spindle with respect to such characteristics as the resting membrane potential (RMP), the parameters of the miniature postsynaptic potentials (MPSP), and the distribution of amplitudes of the MPSPs, on the basis of which certain conclusions could be drawn regarding the type of the fibers studied [2, 7]. Miniature potentials of intrafusal fibers have not hitherto been investigated.

EXPERIMENTAL METHOD

The muscle spindle was isolated from the extensor digiti longus IV muscle in the hind limb of *Rana temporaria*. Experiments were carried out in the fall and spring. The muscle was dissected under the MBS-2 binocular microscope. Extrafusal muscle fibers surrounding the spindle were removed with needles. To facilitate puncture of the capsule of the spindle by the microelectrode, it was treated with 1% hyaluronidase solution (Reanal) for 10 min. The dissected spindle together with fragments of the extrafusal fibers (Fig. 1a) was placed in a chamber containing running physiological saline (NaCl 110 mM, KCl 2.5 mM, NaH_2PO_4 0.85 mM, Na_2HPO_4 2.15 mM, $CaCl_2$ 1.8 mM; pH 7.1-7.4) and rinsed for 1 h to remove the hyaluronidase. Some of the experiments were carried out on the extrafusal muscle fibers of the same muscle in order to compare them with the intrafusal fibers. Potentials were derived intracellularly from the muscle fibers; very thin glass microelectrodes filled with 3 M KCl, with a tip having

Laboratory of Neuromuscular Physiology, Physiological Institute, Leningrad University.
(Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 2, pp. 136-139, February, 1977. Original article submitted August 2, 1976.

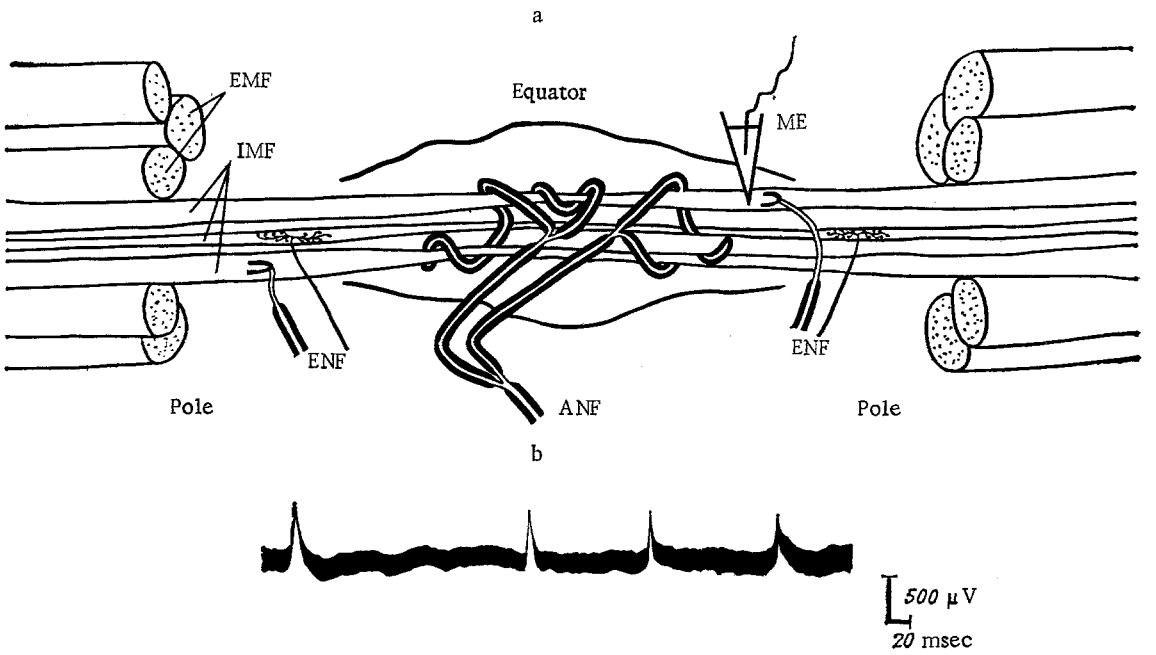

Fig. 1. Scheme showing structure of spindle (a) and example of MPSP recorded from an intrafusal fiber (b). EMF) Extrafusal muscle fibers; IMF) intrafusal muscle fibers; ENF) efferent nerve fibers; ANF) afferent nerve fiber; ME) microelectrode.

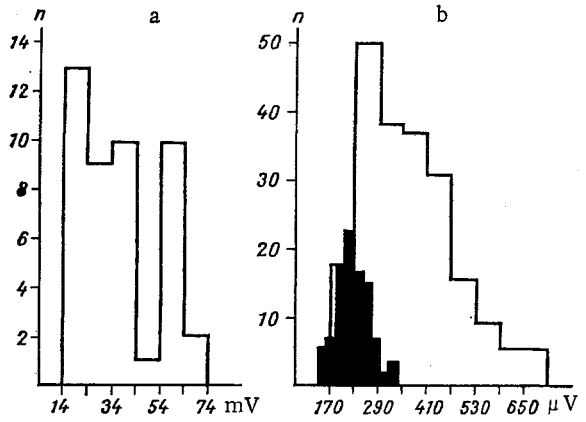

Fig. 2. Histograms of distribution of RMPs and MPSPs of intrafusal muscle fibers of extensor digit longus IV muscle of frog: a) distribution of RMPs among mass of fibers; b) distribution of MPSPs of two fibers (shaded area for fibers with normal distribution, unshaded area for asymmetrical distribution). Ordinate, number of data; abscissa, values of RMP (a) and MPSP (b).

a resistance of 30-80 MΩ, were used. Signals were amplified by the UBP-1-02 amplifier and recorded on the N-115 loop oscillograph. The S1-19B cathode-ray oscilloscope was used for visual control. RMPs and MPSPs were recorded. Altogether 70 intrafusal and 100 extrafusal fibers were investigated. The results were subjected to statistical analysis by the method of asymmetry in order to assess the asymmetry of distribution of MPSP amplitudes, which was expressed differently in the fibers of different types. The coefficient of asymmetry t_A was calculated; the distribution was regarded as significantly asymmetrical if $t_A > 3$.

EXPERIMENTAL RESULTS

Proprioceptors of the muscle used are known to have the shape of tandems — chains of successive spindles [3]. The intrafusal muscle fibers in these tandems vary in length from

TABLE 1. Distribution of Amplitudes of MPSPs of Frog Intrafusal Muscle Fibers

No. of experiment	Region of recording	RMP, mV	Number of data	MPSP, μV (M± m)	Coefficients of variation	Coefficients of asymmetry
26 (1)	Pole	—	200	245,1±6,4	0,37	8,8
26 (2)	"	43	57	310,8±21,5	0,52	4,3
28 (1)	"	31	150	125,0±2,1	0,20	1,3
29 (2)	"	14	95	225,3±4,2	0,18	1,4
29 (3)	Equator	37	200	581,8±15,7	0,38	4,6
36 (1)	"	43	117	906,4±26,0	0,31	8,5
42 (1)	"	57	97	1404,4±47,3	0,33	1,2

1 mm to the length of the whole tandem, approximately equal to the length of the muscle. When the electrophysiological characteristics are referred to later, characteristics of those regions of the intrafusal fibers located not more than 1.5 mm from the microelectrode, or about 2λ, will be implied [10].

The RMPs of the intrafusal muscle fibers tested varied from 14 to 73 mV and had a relatively small mean value (37 ± 2.6 mV, σ = 17.5). The distribution of RMPs of the mass of fibers was bimodal (Fig. 2a). The peak in the region of lower values of RMP probably corresponded to fibers with a small diameter, which are severely damaged even by very thin microelectrodes. The presence of these very thin muscle fibers (diameter 3-7 μ) in the intrafusal bundle has been demonstrated histologically [3, 6]. The possibility cannot be ruled out that this group also contains fibers with low RPM values from the very beginning, especially tonic fibers. The second peak corresponds to RMPs usually recorded in the intrafusal bundle [5, 8].

MPSPs could not be recorded from all intrafusal fibers. However, satisfactory records of MPSPs were obtained from 13 fibers in the equatorial and polar regions of the spindle (Fig. 1b). The mean frequency of the MPSPs was 6 ± 0.2 per second (σ = 2.4). Incidentally, the diameter of the intrafusal fibers is greater at the pole and less at the equator [9]. This is reflected in the amplitude of the MPSP, for the smaller the diameter of the fiber, the greater its input resistance and the greater the amplitude of the MPSP. The mean amplitude of MPSPs at the pole was 236.7 ± 8.7 μV, and at the equator 964.2 ± 29.7 μV. Sample data for the amplitudes of the MPSPs are given in Table 1.

Analysis of the distribution of amplitudes of MPSPs for each fiber, using the asymmetry method, showed that several fibers (see Table 1: experiment 26, experiment 29, fiber 3, and experiment 36) had an asymmetrical distribution of MPSP amplitudes ($t_A \gg 3$) and a high coefficient of variation of the MPSP amplitudes, characteristic of fibers with multiple innervation (Fig. 2b). Fibers with a normal distribution of MPSP amplitudes ($t_A < 3$) and with a low coefficient of variation of MPSP amplitudes, characteristic of fast fibers with single innervation, also were found (see Table 1: experiment 28 and experiment 29, fiber 2) (Fig. 2b).

One fiber (Table 1, experiment 42), with wide limits of distribution of MPSP amplitudes, with a high coefficient of variation of amplitudes, but at the same time, with a low value of the coefficient of asymmetry (t_A = 1.2), was found among the mass of intrafusal fibers studied. This nonasymmetrical form of distribution of MPSP amplitudes can evidently be explained by coexistence of innervation typical of both fast and slow muscle fibers in this particular fiber. As a result, the asymmetrical and normal distributions were superposed, thus diminishing the asymmetry.

The mean value of the RMPs of the extrafusal muscle fibers tested was 51 ± 16.6 mV. The frequency of the MPSPs was a little higher than for the intrafusal fibers: on average 10 ± 0.2 per second (σ = 2.2). The upper limits of distributions of MPSP amplitudes were lower for these than for the intrafusal fibers. The reason is that the diameter of the extrafusal fibers is on average an order of magnitude higher than that of the intrafusal fibers. Judging from the distribution of MPSP amplitudes, two types of fibers also were present among the extrafusal fibers: those with single innervation (phasic) and those with multiple innervation (tonic).

The experiments thus showed that intrafusal fibers or, more exactly, those parts of them which belong to the same sensory region of the tandem, are similar in their electrophysiological characteristics to extrafusal fibers. The fiber composition of the intrafusal fibers

is heterogeneous and includes fibers with single innervation (phasic), with multiple innervation (tonic) and, probably, fibers combining both types of innervation also.

LITERATURE CITED

1. D. P. Matyushkin, The Oculomotor Apparatus of Mammals [in Russian], Leningrad (1972).
2. D. P. Matyushkin and T. M. Drabkina, Fiziol. Zh. SSSR, No. 4, 541 (1973).
3. D. Barker and M. Cope, J. Anat. (London), 96, 49 (1962).
4. D. Barker, M. J. Stacey, and M. N. Adal, Phil. Trans. Roy. Soc. B, 258, 315 (1970).
5. C. Eyzaguirre, J. Neurophysiol., 20, 523 (1957).
6. E. G. Gray, Proc. Roy. Soc. B, 146, 416 (1957).
7. A. Hess and G. Pilar, J. Physiol. (London), 169, 780 (1963).
8. F. Ito, N. Kanamori, and H. Kuroda, Nature, 249, 69 (1974).
9. U. Jahn, Acta Anat. (Basel), 39, 341 (1959).
10. K. Koketsu and S. Nishi, J. Physiol. (London), 139, 15 (1957).