starting from 37°C (Fig. 2). Assessment of the relative changes in the force of contractions of the heart muscle of a warm-blooded animal (cat) during stretching or during an increase in the frequency of its stimulation thus convincingly proves that functioning of the Starling mechanism is independent of temperature and that the chrono-inotropic mechanism is inhibited by cold.

Considering that differences in the temperature sensitivity of biological phenomena to some extent reflect dissimilarity between the processes determining these phenomena [5], it can be concluded that the results of the present investigation also indicate differences in the nature of the processes lying at the basis of these two mechanisms of autoregulation of contractility. This confirms existing views that the predominant factor in the realization of the Starling mechanism is a physical process of change in the spatial mutual arrangement of the actin and myosin filaments, which is independent of temperature, whereas the predominant factor in the realization of the chrono-inotropic mechanism is chemical reactions in the process of electromechanical coupling, which are inhibited by cold.

LITERATURE CITED

1. O. A. Zhigal'skii and V. Ya. Izakov, Izv. Akad. Nauk SSSR, Ser. Biol., No. 2, 281 (1977).
2. V. M. Pokrovskii, Yu. R. Sheikh-Zade, V. V. Vovereidt, et al., Élektron. Tekh., Ser. 4, No. 7, 91 (1979).
3. P. M. Starkov and Yu. R. Sheikh-Zade, Fiziol. Zh. SSSR, No. 6, 949 (1972).
4. V. V. Vovereidt and Yu. R. Sheikh-Zade, Abstracts Deposited with the All-Union Institute of Scientific and Technical Information, No. 3649-79 (1979).
5. V. M. Pokrovskii, Byull. Éksp. Biol. Med., No. 8, 9 (1969).
6. J. R. Blinks and J. Koch-Weser, Pharmacol. Rev., 15, 531 (1963).
7. V. Kruta and J. Stejskalova, Arch. Int. Physiol. Biochem., 68, 152 (1960).
8. M. L. Kahn, F. Kavaler, and V. J. Fisher, Am. J. Physiol., 230, 431 (1976).
9. S. Hajdu and A. Szent-Gyorgyi, Am. J. Physiol., 168, 159 (1952).
10. G. A. Langer and A. J. Brady, J. Gen. Physiol., 52, 682 (1969).
11. M. Kababgi, W. Westphal, and E. Bauereisen, Basic Res. Cardiol., 69, 47 (1974).

NONQUANTUM ACETYLCHOLINE RELEASE IN A NERVE-MUSCLE PREPARATION OF DYSTROPHIC 129/Rej MOUSE DIAPHRAGM

E. M. Volkov and G. I. Poletaev

UDC 616.74-009.54-055.5/.7-092.9-07: 616.26-018.6

KEY WORDS: congenital muscular dystrophy; nonquantum acetylcholine release; skeletal muscle.

A line of mice (129/Rej) is known which has a recessive genetic defect, manifested in the homozygous state as a lesion of the skeletal muscle, comparable in its features with the changes in human skeletal muscle in congenital muscular dystrophy, described as Duchenne's syndrome [5]. Some muscle fibers of these homozygous dystrophic mice are characterized in particular by extrasynaptic sensitivity to acetylcholine (ACh), resistance of their action potentials to tetrodotoxin, and lower values of resting membrane potential (RMP) than muscle fibers of phenotypically normal individuals [5, 7]. Such changes in the properties of the muscle membrane are known to be characteristic of denervated muscle fibers [1]. In this connection it has been suggested that the existing genetic defect is expressed ultimately as a disturbance of neurotrophic control of the muscle fibers [1, 5].

However, the affected muscle fibers of dystrophic mice are not excluded from motor activity and are indistinguishable from intact muscle fibers in the character of both quantum-

Department of Biology, Kazan' Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 8, pp. 33-35, August, 1983. Original article submitted October 11, 1983.

0007-4888/83/9608-1064$07.50

TABLE 1. RMP of Muscle Fibers of Affected and Phenotypically Normal 129/Rej Mice in Synaptic (A) and Extrasynaptic (B) Regions after Treatment of the Muscle with Armin, before and after Addition of D-Tubocurarine Chloride

Experimental conditions	Homozygous sick		Phenotypically normal	
	A	B	A	B
Treatment with armin	74,1±1,0 (52) $P<0,01$	75,3±1,0 (53)	79,4±1,0 (55)	82,0±1,0 (85)
Addition of D-tubocurarine chloride	80,7±0,9 (45) $P<0,01$	77,0±1,1 (45)	85,9±0,9 (51) $P<0,01$	83,1±1,1 (52)

Legend. Mean values and standard errors are shown. Number of fibers tested given in parentheses. Level of significance taken as 0.05 by Student's t test.

induced and spontaneous ACh secretion from motor nerve endings [4, 6]. Accordingly it is not quite clear what is primary: the defect of the genome of the muscle fibers making them insusceptible to neurotrophic influences, or a lesion of the nervous system, preventing trophic influences on the target. The latter hypothesis does not contradict the fact that neuromuscular transmission is undisturbed, for we know that the nervous system exercises neurotrophic control quite apart from any direct dependence on its ability to conduct excitation [1].

Besides quantum ACh secretion, nonquantum ACh release has also been shown to exist [9, 10], and hypothetically it may have a role in the mechanism of neurotrophic control of muscle fibers [8].

It was accordingly decided to study the character of nonquantum ACh secretion from motor endings, which is important both to a description of the complete picture of this type of muscular dystrophy in mice, and also for an understanding of the general physiological mechanisms of neurotrophic control of skeletal muscles in vertebrates, including in man. The investigation described below was undertaken to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on the diaphragm of homozygous sick and phenotypically normal 129/Rej mice of the same litter, 2.5 months old. The phenotypically normal mice acted as the control. RMP were recorded in synaptic and extrasynaptic regions of the same fibers intercellularly by means of the standard microelectrode technique. The criterion that the microelectrode was in the zone of synaptic contact was the recording of miniature end-plate potentials in this case.

Nonquantum ACh release from nerve endings was estimated from the level of hyperpolarization of the muscle membrane in the synaptic zone, developing after application of curarine and inhibition of acetylcholinesterase [9]. By using as test object the mouse diaphragm, hyperpolarization responses (HR) whose amplitude characteristics can be subjected to statistical analysis, can be obtained [3, 10].

During the experiments the isolated muscle was kept in continuously flowing Ringer's solution of the following composition (in mM): NaCl 120, KCl 5.0, $CaCl_2$ 2.0, $MgCl_2$ 1.0, NaH_2PO_4 1.0, $NaHCO_3$ 24.0, glucose 17.0, pH 7.2, at 30°C, with oxygenation of the solution with a mixture of 95% O_2 and 5% CO_2.

Before the experiment the muscle was kept for 30 min in Ringer's solution containing armin (USSR origin) $5 \cdot 10^{-5}$ g/ml, an irreversible acetylcholinesterase inhibitor, after which the preparation was rinsed for 20 min. RMP of the muscle fibers was measured immediately after rinsing in ordinary Ringer's solution and in Ringer's solution containing 10^{-4} g/ml D-tubocurarine chloride (from Orion, Finland).

EXPERIMENTAL RESULTS

The experiments showed that RMP in the synaptic zone of phenotypically normal mice, used as the control, after treatment of the muscle with armin was the same as in the extrasynaptic region of the same muscle fibers (Table 1). Addition of D-tubocurarine chloride to the solution did not change the values of RMP of the muscle fibers in the extrasynaptic zone (Table 1), whereas this induced hyperpolarization of the postsynaptic membrane on average by 6.5 ± 1.2 mV compared with the original values.

RMP of the muscle fibers of the homozygous dystrophic mice was significantly lower than that of phenotypically normal mice of the same litter (Table 1). This fact confirms previous observations [5] and indicates that these changes are denervation-like in character [1].

RMP in the synaptic region of the muscle fibers of the homozygous dystrophic mice, just as in the control, was the same as in the extrasynaptic region of the same fibers (Table 1). Addition of D-tubocurarine chloride to the solution did not change RMP of the extrasynaptic muscle membrane, but hyperpolarized the postsynaptic membrane of the muscle fibers on average by 6.6 ± 1.4 mV (Table 1).

It must be pointed out that the values of RMP of muscle fibers in a mouse after denervation are lower than those which we recorded in dystrophic mice. This fact can be explained on the grounds that not all muscle fibers of homozygous sick mice are affected to the same degree, and some of them remain healthy. By the method which we used, the test population of muscle fibers included both normal and defective fibers, so that as a result intermediate values of RMP were obtained. This same state of affairs could also affect estimation of nonquantum release of ACh from nerve endings of the dystrophic mice. It is impossible to select only affected fibers in the course of the experiment and to estimate nonquantum ACh release only in them, by using the value of RMP as the criterion, because of the great variability of RMP values both in conjecturally normal and in dystrophic muscle fibers. No useful purpose can be served by estimating the value of HR to applied D-tubocurarine chloride by recording RMP continuously before and after hyperpolarization of a single fiber, for the membrane depolarization which develops rapidly in this case leads to even greater errors when the true value of HR is estimated.

The method used to collect the data in the present experiments thus enables a difference in the level of RMP of the muscle fibers in sick and phenotypically normal mice to be detected, and it is also clear that the test population of muscle fibers contains an adequate number of affected fibers. In addition, the value of HR itself was high enough. All these facts suggest that if the value of HR in dystrophic fibers in response to application of D-tubocurarine chloride differed significantly from the control, this difference could have been detected by the method used to collect the data.

As the experimental results showed, the value of HR in the diaphragm of phenotypically normal and homozygous dystrophic mice was practically identical.

Consequently there are no grounds for considering that nonquantum ACh release from motor nerve endings of dystrophic muscle fibers may be substantially affected, and can thus be the main cause of the denervation-like depression of RMP of the muscle fibers which we recorded in dystrophic 129/Rej mice.

LITERATURE CITED

1. E. M. Volkov and G. I. Poletaev, Usp. Fiziol. Nauk, 13, No. 3, 9 (1982).
2. E. M. Volkov and G. I. Poletaev, Byull. Éksp. Biol. Med., No. 10, 18 (1982).
3. V. A. Voronin, E. E. Nikol'skii, and F. Vyskocil, in: Proceedings of the 1st All-Union Biophysical Congress [in Russian], Moscow (1982), pp. 108-109.
4. S. Carbonetto, J. Neurophysiol., 40, 836 (1977).
5. L. W. Duchen and E. Stefani, J. Physiol. (London), 212, 535 (1971).
6. J. B. Harris and L. Ribchester, J. Physiol. (London), 296, 245 (1979).
7. R. R. C. Howe, J. A. Telefer, and B. G. Livett, Exp. Neurol., 56, 42 (1977).
8. D. A. Mathers and S. Thesleff, J. Physiol. (London), 282, 105 (1978).
9. B. Katz and R. Miledi, Proc. R. Soc. London, B, 196, 59 (1977).
10. F. Vyskocil, Prog. Brain Res., 49, 183 (1979).