SYNAPTIC STRUCTURE AND PARAMETERS OF THE ELECTRICALLY EXCITABLE MEMBRANE OF MUSCLE FIBERS IN FETAL AND NEONATAL RABBITS

M. G. Nemets, G. V. Bryukhin, and N. V. Darinskii

UDC 612.64"74

With strengthening of adaptive motor responses of rabbit fetuses during mild inhibition of the "gestation dominant" development of the synaptic structure and corresponding characteristics of the muscle fibers is accelerated. During inhibition of fetal motor responses associated with sharp inhibition of the gestation dominant, development of the synaptic structure, electrophysiological characteristics, and cross striation of the muscle fibers is retarded; this is regarded as the result of a deficiency of neurotrophic influences on the skeletal muscle in physiologically immature fetal and neonatal animals.

KEY WORDS: *cholinesterase; muscle potentials; fetal development.*

Attention was directed earlier to the high scatter of the membrane potential of skeletal muscle fibers and of other physiological parameters in animals in the early postnatal period depending on the degree of their physiological maturity [1, 3, 6, 7, 11].

The character of formation of synaptic structure and properties of the electrically excitable membrane of muscle fibers were investigated in fetal and neonatal rabbits during inhibition of the "gestation dominant."

EXPERIMENTAL METHOD

Fetuses resulting from a normal pregnancy were included in group 1. Intrauterine development of the fetuses of this group was characterized by a normal course of adaptive motor responses. Group 2 included fetuses whose intrauterine development took place under conditions of mild inhibition of the gestation dominant, as a result of which the frequency and intensity of adaptive motor responses were increased. Group 3 included fetuses whose intrauterine development took place during severe inhibition of the gestation dominant. Adaptive motor responses in the fetuses of this group were suppressed.

The fetuses were taken by caesarian section at different times of pregnancy starting from the 21st day. Inhibition of the gestation dominant was produced by the methods described previously [2, 4, 9, 12]. The biceps brachii muscle was used as the test object. The character of the synaptic structure was judged from the results of staining for acetylcholinesterase (ACE) [8] and nonspecific esterase (NE) [5]. The membrane potential (MP) and electrical response to direct stimulation were measured by

Laboratory of Age Physiology and Pathology, Institute of General Pathology and Pathophysiology, Academy of Medical Sciences of the USSR, Moscow. Department of Histology and Embryology, Chelyabinsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 8, pp. 15-18, August, 1975. Original article submitted September 6, 1974.

intracellular glass microelectrodes filled with 3 M KCl solution [6, 11], with a tip under 1 μ in diameter and a resistance of 30-60 MΩ. The measurements were made without anesthesia, on the securely fixed forelimb. The body temperature and the temperature of the biceps muscle were maintained at 36-37°C. The absolute and relative weight of the total muscle mass and of the biceps muscle alone were determined. Altogether 80 normal fetuses and newborn animals and 120 fetuses and newborn animals with inhibition of the gestation dominant were used.

EXPERIMENTAL RESULTS AND DISCUSSION

Histochemical preparations from fetuses on the 21st day of normal pregnancy showed areas of generalized distribution of ACE and NE in the form of pale granules and patches without clearly defined borders. On the 25th day and, in particular, on the 27th day of pregnancy the zones of synaptic ACE and NE acquired a tendency toward concentration in the region of contact of the axon terminals with the muscle fiber. On either side of these zones, a diffuse, generalized distribution of ACE and NE could still be observed (Figs. 1a, b and 2a). At this age, the value of MP in the muscles of the forelimbs (Table 1), it will be noted, is higher than in the hindlimb muscles [6]. Toward the end of intrauterine development (29-30 days) and in the newborn rabbits the synaptic zone was more concentrated still. Its area increased. At the same time, the diffuse character of the paler staining still remained, although the number of granules was reduced (Figs. 1b and 2b). Some increase in MP was observed in this period. The electrical response to direct stimulation at this time showed the typical features of the end-plate potential of adult animals or the local potential (LP), and did not exceed the MP in value. This response was recorded throughout the extent of

TABLE 1. Weight and Electrophysiological Characteristics of Skeletal Muscles of Rabbit Fetuses under Different Conditions of Intrauterine Development (M ± m)

Age (in days)	Group of animals	Weight of fetus (in g)	Relative weight of total muscle mass (in %)	Relative weight of biceps muscle (in %)	Membrane potential (in mV)	Amplitude of evoked potential (in mV)	Duration of evoked potential (in msec)
23	1	8,4±0 27	—	0,32±0,06	28,2±3,8	—	—
29	1	42,0±1,1	21,3±3,7	0,41±0,07	31,3±3,4	26,4±4,2	3,4±0,8
29	2	55,4±0,9	25,9±2,6	0,52±0,04	35,8±0,9	35,0±2,4	3,2±0,6
29	3	24,7±1,0	17,6±5,1	0,35±0,02	26,3±1,7	19,5±3,9	5,7±1,4
First day after birth	1	50,0±9,1	23,2±2,0	0,43±0,25	32,5±1,47	30,8±2,9	3,5±0,9
	2	58,3±2,2	26,2±3,5	0,54±0,08	37,3±1,1	37,6±3,9	3,1±1,0
	3	34,0±1,9	20,0±4,2	0,31±0,07	30,1±1,9	25,7±3,2	5,1±1,2

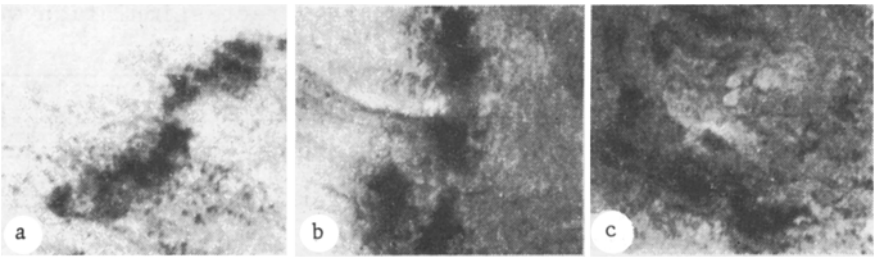

Fig. 1. Acetylcholinesterase activity of fibers of biceps brachii muscle in fetus on 21st day of intrauterine development (a) and in newborn rabbits — physiologically mature (b) and immature (c).

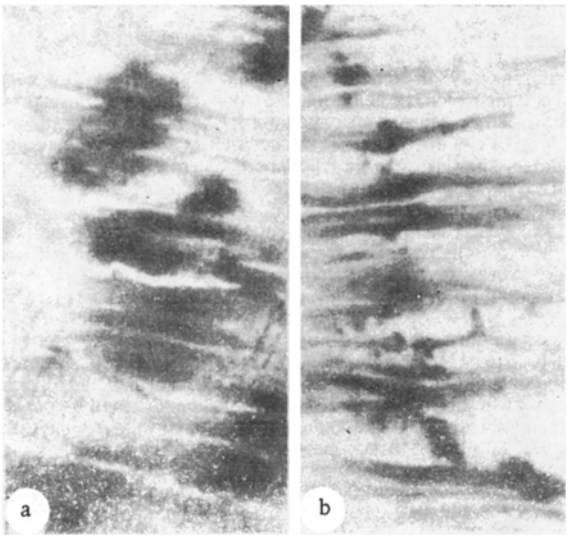

Fig. 2. Nonspecific esterase of biceps brachii fibers of newborn rabbit — physiologically mature (a) and immature (b).

the muscle fiber. Its comparatively small value can be explained by the small diameter of the muscle fibers at this age.

In fetuses at the end of intrauterine development and the newborn rabbits of group 2, the concentration, centralization, and clarity of outline of the synaptic zone were more marked, whereas the area of zone ACE was greater than in the fetuses and newborn rabbits of group 1. MP and LP of the muscle fibers of the animals of group 2 were higher and the duration of LP was shorter than in the animals of group 1. The relative weights of the total muscle mass and of the biceps muscle itself also were greater in group 2.

In fetuses at the end of pregnancy and newborn rabbits of group 3 (physiologically immature) the synaptic zone remained generalized throughtout the length of the muscle fibers; in a few cases it was actually more diffuse than in muscles of normal fetuses on the 21st day of intrauterine development (Fig. 1c). The values of MP and LP in the animals of group 3 were lower, whereas the duration of LP was greater than in the fetuses and newborn rabbits of group 1 and, more especially, group 2. In the rabbits of group 3 the relative weight of the total muscle mass and of the biceps muscle was significantly lower. Cross striation of the muscle fibers was less marked in the fetuses and newborn rabbits of this group than in the animals of groups 1 and 2.

These results suggest that intensification of adaptive motor responses in the fetus and the associated more rapid flow of impulses from the centers of innervation of the skeletal muscle are factors which accelerate development of the synaptic structure and membrane properties of muscle fibers. Inhibition of activity of the centers of innervation of skeletal muscle associated with inhibition of the gestation dominant can be attributed to changes produced in them by acid metabolic products [2, 9, 10]. The results obtained with the fetuses and newborn rabbits of group 3 can thus naturally be regarded as an expression of inhibition of neurotrophic influences on skeletal muscle.

LITERATURE CITED

1. T. A. Adzhimolaev, "Electrophysiological characteristics of formation and transformation of activity of the neuromuscular system during postnatal ontogeny of mammals," Author's Abstract of Candidate's Dissertation (1963).
2. I. A. Arshavskii, Outlines of Age Physiology [in Russian], Moscow (1967).
3. I. A. Arshavskii, Uspekhi Fiziol. Nauk, 2, No. 4, 100 (1971).
4. I. A. Arshavskii, M. Nemets, and Z. F. Surovtseva, Vest. Akad. Med. Nauk SSSR, No. 11, 60 (1962).
5. M. Burstone, Enzyme Histochemistry [Russian translation], Moscow (1965).
6. N. V. Darinskii, "Analysis of structural and energetic parameters of skeletal muscles of mammals in connection with neurotrophic regulation in ontogeny," Author's Abstract of Candidate's Dissertation, Moscow (1974).
7. N. V. Darinskii and Z. F. Surovtseva, Byull. Éksperim. Biol. i Med., No. 2, 11 (1974).
8. R. Lillie, Pathohistological Techniques and Practical Histochemistry [Russian translation], Moscow (1969).
9. M. G. Nemets, "Analysis of mechanisms determining normal and abnormal intrauterine development," Author's Abstract of Candidate's Dissertation, Moscow (1964).

10. M. G. Nemets and I. S. Tarasova, Byull. Éksperim. Biol. i Med., No. 8, 35 (1974).
11. S. S. Solomatin, Zh. Évolyuts. Biokhim. i Fiziol., No. 4, 30 (1967).
12. Z. S. Surovtseva, "Characteristics of growth and development of rabbit fetuses during experimental disturbance of pregnancy," Author's Abstract of Candidate's Dissertation, Moscow (1969).