LITERATURE CITED

1. L. L. Katalymov, Neirofiziologiya, No. 5, 532 (1974).
2. L. L. Katalymov, Neirofiziologiya, No. 2, 208 (1975).
3. L. L. Katalymov, Byull. Éksp. Biol. Med., No. 3, 3 (1975).
4. R. Lorente de No, A Study of Nerve Physiology, New York (1947).

ELECTROPHYSIOLOGICAL, MORPHOMETRIC, AND HISTOCHEMICAL CHARACTERISTICS OF MUSCLE FIBERS OF THE FROG SARTORIUS MUSCLE

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Investigation of succinate dehydrogenase (SD) activity in the fibers of the frog sartorius muscle revealed three groups of muscle fibers (dark, pale, and intermediate). The SD activity in the muscle fibers was inversely proportional to their diameter. The outer surface of the sartorius muscle consists mainly of dark muscle fibers, the inner surface mainly of pale fibers. A microelectrode study showed that fibers of the outer surface have lower values of action potentials, a longer negative afterpotential, a lower quantum composition of the end-plate potentials, and higher amplitude and lower frequency of miniature end-plate potentials than fibers of the inner surface. Analysis of the results reveals definite relations between the histochemical profile of frog sartorius muscle fibers and their electrophysiological characteristics.

KEY WORDS: frog sartorius muscle; types of muscle fibers; succinate dehydrogenase; electrophysiological differences.

Three types of muscle fibers are distinguished in the amphibian motor system: phasic, tonic, and intermediate. Phasic fibers are characterized by a high transmembrane potential (TMP), by generation of an action potential (AP) in response to single stimulation of the motor nerve, by a short latent period and high quantum composition of synaptic potentials [4, 5], by weak summation of synaptic potentials in response to repetitive stimulation [4-6], and by other electrophysiological features. Phasic muscle fibers also differ from tonic and intermediate by their greater diameter [7, 9], the morphology of the motor nerve endings (endings of the "end brush" type), low activity of oxidative enzymes [9, 11], and various other structural and histochemical features.

The frog sartorius muscle is a typical phasic muscle, all fibers of which are regarded as functionally homogeneous. However, recent observations indicate that the fibers of the sartorius muscle vary in amplitude and quantum composition of their end-plate potentials (EPP), the dynamics of amplitude of EPP during repetitive stimulation[1], the frequency of their miniature end-plate potentials (MEPP), the duration of their AP [3], the area of synaptic contact [10], and activity of their oxidative and glycolytic enzymes [9].

The question naturally arises whether electrophysiological differences between muscle fibers of the sartorius muscle are connected with morphological and histochemical heterogeneity. The following investigation was carried out to study this problem.

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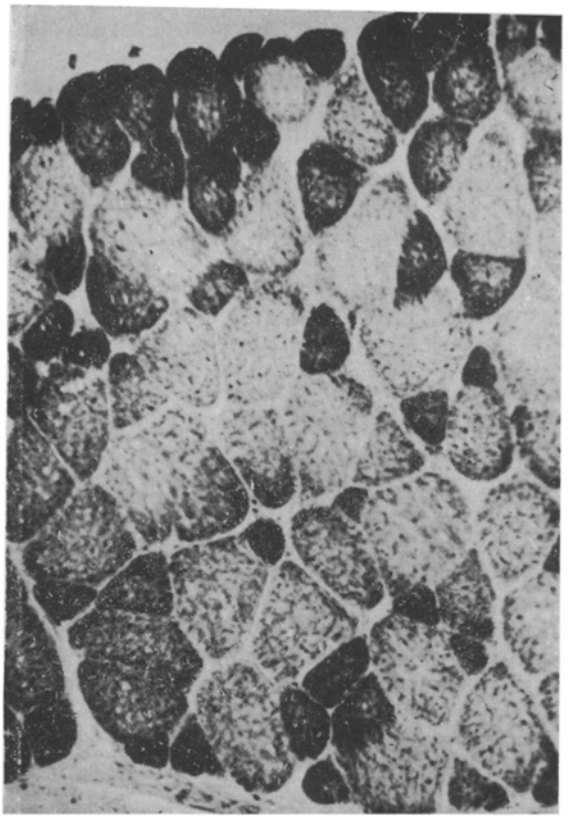

Fig. 1. SD activity in fibers of frog sartorius muscle. Outer surface above, inner surface below. Transverse section through sartorius muscle. Nitro-BT, 63×.

EXPERIMENTAL METHOD

Experiments were carried out on nerve-muscle preparations of the sartorius muscle and sciatic nerve of *Rana ridibunda*. The nerve-muscle preparation was kept in a chamber with a capacity of 14 ml, the rate of flow in which could be controlled at a constant level. Standard Ringer's solution (pH 7.2-7.4) was used. The experiments were carried out at 18-20°C. AP of the muscle fibers were blocked by the addition of D-tubocurarine to the Ringer's solution in a concentration of $1 \cdot 10^{-6}$ g/ml or by cutting the muscle fibers transversely [2]. The technique of the microelectrode investigations was fully described by the writers previously [1]. Succinate dehydrogenase (SD) activity was determined by the nitro-BT method followed by densitometry of the negatives on the IFO-451 recording microdensitometer and by measurement of the areas of cross section of the muscle fibers from photographic prints.

EXPERIMENTAL RESULTS

The SD activity, as judged by the area of deposition of the histochemical reaction product, was characterized by considerable variability in the different muscle fibers (Fig. 1). On the basis of this feature, all fibers of the muscle were divided into three groups: fibers with weak activity (pale fibers), with moderate (intermediate), and with high activity of the enzyme (dark fibers). According to this working classification muscle fibers located on the outer surface of the muscle were of the dark type (Fig. 1, Fig. 2B, graph I), and fibers lying on the inner surface were mainly of the pale and intermediate types (Fig. 1, Fig. 2B, graph II). Investigation of sections of the sartorius muscle showed considerable differences in the diameters of the muscle fibers (from 30 to 190 μ). Small muscle fibers (30-100 μ in diameter) lay on the outer surface of the muscle (Fig. 2A, graph I) and large fibers (diameter 40-190 μ) on the inner surface (Fig. 2A, graph II). All three groups of fibers were present in the depth of the muscle, but they were mainly pale and intermediate in type (Fig. 2C). Dark fibers accounted for 36.9 ± 1.8% of the total number of fibers in the sartorius muscle. Fibers with moderate and weak staining accounted for 14.9 ± 1.6 and 48.2 ± 2.2%,

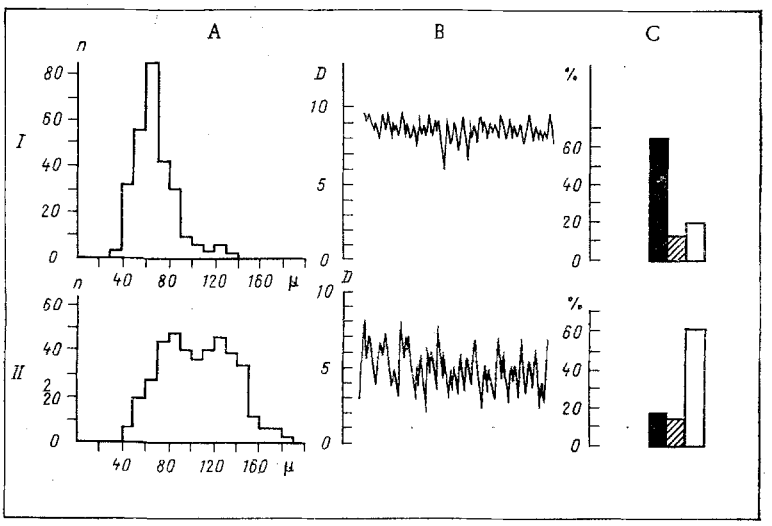

Fig. 2. Morphometric and histochemical characteristics of muscle fibers of outer and inner surfaces of frog sartorius muscle. I) Outer surface; II) inner surface. A) Histograms of distribution of muscle fibers by diameter. Abscissa, diameter of muscle fibers (in μ); ordinate, number of determinations. B) Densitograms of sartorius muscle (D — conventional units of optical density). C) Relative content (in %) of dark (black columns), intermediate (shaded columns), and pale (unshaded columns) muscle fibers.

respectively. Measurement of the area of cross section of the muscle fibers showed that dark fibers had the smallest area (2500.0 ± 49.4 μ^2) and pale muscle fibers the largest area (6497.3 ± 84.8 μ^2). The intermediate fibers had an area of cross section in between (5182.0 ± 96.0 μ^2). Strong correlation ($r = -0.817$, $P < 0.001$) was found between the features studied (area of cross section of the muscle fibers and their optical density).

The inner surface of the sartorius muscle thus consists mainly of large muscle fibers with a weak reaction for SD (pale), whereas the outer surface consists mainly of small muscle fibers staining strongly for SD (dark). This is a very convenient phenomenon for the separate electrophysiological investigation of muscle fibers that differ in their morphological and histochemical characteristics.

TMP in the fibers of the outer and inner surfaces of the muscle was 67.0 ± 9.1 and 65.6 ± 8.3 mV, respectively. Altogether 40.1% of the fibers studied on the outer surface of the muscle and 50.6% on the inner surface responded to direct single stimulation by a spreading AP. In response to paired stimulation with an interval of 10-20 msec all muscle fibers on the outer and inner surfaces of the muscle responded by AP development. The amplitude of the AP in the fibers on the outer surface often (in 23.5% of cases) did not exceed the resting potential, whereas in fibers on the inner surface AP with marked overshoot always were recorded. After-depolarization lasted longer in the fibers on the outer surface than in those on the inner surface (Fig. 3A). The half-decay time of the after-potential was 10.2 ± 1.5 and 7.9 ± 0.8 msec, respectively. The duration of the depolarization phase and the half-decay time of the peak were the same in fibers on the outer and inner surface of the muscle.

The frequency of MEPP in fibers of the outer surface was 0.542 ± 0.114 spikes/sec and the amplitude of MEPP was 0.393 ± 0.020 mV, whereas in fibers on the inner surface the corresponding values were 2.235 ± 0.358 spikes/sec and 0.230 ± 0.014 mV (Fig. 3B). Dispersion of the amplitude of MEPP in the fibers on the outer surface was considerably greater than in fibers on the inner surface (Fig. 3C).

The latent period of EPP in the fibers on the outer surface of the muscle was 3.09 ± 0.04 msec and in fibers on the inner surface 2.40 ± 0.04 msec. After curarization the quantum composition of EPP in fibers of the outer surface of the muscle (51.2 ± 10.4 quanta) was

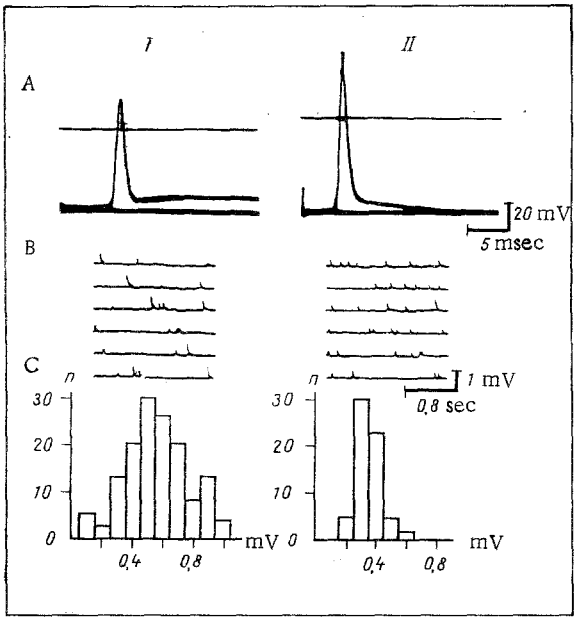

Fig. 3. Electrophysiological characteristics of muscle fibers on outer and inner surfaces of frog sartorius muscle. I) Outer surface, II) inner surface. A) Action potentials; B) MEPP; C) histograms of distribution of MEPP amplitudes. Abscissa, amplitude of MEPP (in mV); ordinate, number of observations. Each histogram plotted from results of one experiment.

considerably lower than in fibers on the inner surface (198.0 ± 50.2 quanta). During repetitive stimulation marked and prolonged potentiation of the amplitude of EPP was observed in these fibers. Depending on the quantum composition of their EPP, all the synaptic formations in the sartorius muscle were subdivided into three types [1]: type I — EPP consisting of over 200 quanta, type II — 100-200 quanta, and type III — under 100 quanta. The distribution of synapses into three types in nerve-muscle preparations whose AP had been blocked by transverse incision of the muscle fibers from the outer surface was: type I — 4%, type II — 35%, type III — 61%, and in fibers of the inner surface: type I — 65%, type II — 31%, type III — 4%. Consequently, compared with the inner surface, the outer surface of the sartorius muscle consists of muscle fibers with relatively low quantum composition of their EPP, low frequency and high amplitude of their MEPP, and more prolonged after-depolarization of AP.

The morphometric and histochemical investigation thus showed that fibers described as dark constitute the overwhelming majority on the outer surface of the sartorius muscle (65.7± 2.7%), whereas on the inner surface (the one usually used for electrophysiological investigations) most of the muscle fibers (62.3 ± 4.5%) were pale. Electrophysiological studies showed that fibers on the outer surface of the muscle have mainly a low quantum composition of their EPP, i.e., that most fibers here have type III synapses (61%), whereas fibers on the inner surface of the muscle have mainly type I synapses (65%) with, as a rule, a high quantum composition of their EPP. Pale fibers differ from dark in having an AP of high amplitude, a short negative after-potential, a high quantum composition of their EPP, and low amplitude and high frequency of MEPP. The longer latent period of EPP in fibers of the outer surface of the muscle is evidence rather that the large (pale) muscle fibers are innervated by thicker motor axons than the small (dark) fibers.

It is still too early to draw an analogy between the pale and dark muscle fibers in the frog sartorius muscle, on the one hand, and the twitch and slow phasic fibers in mammals, on the other hand. However, some of the distinguishing features of the dark muscle fibers of the frog sartorius muscle (small diameter, high SD activity, relatively low quantum composition of EPP, low values of AP) are also characteristic of the slow muscle fibers of warm-blooded animals [8, 9] and they make such an analogy highly probable.

LITERATURE CITED

1. I. N. Volkova, A. L. Zefirov, E. E. Nikol'skii, et al., Fiziol. Zh. SSSR, 62, 406 (1976).

2. I. N. Volkova, E. E. Nikol'skii, and G. I. Poletaev, Fiziol. Zh. SSSR, 61, 1433 (1975).
3. D. P. Matyushkin and T. M. Drabkina, Biol. Nauki, No. 11, 35 (1971).
4. G. A. Nasledov, Fiziol. Zh. SSSR, 50, 1342 (1964).
5. G. A. Nasledov, Biofizika, 10, 634 (1965).
6. G. A. Nasledov and V. V. Fedorov, Fiziol. Zh. SSSR, 52, 757 (1966).
7. N. F. Skorobovichuk and N. A. Chizhova, Fiziol. Zh. SSSR, 57, 1275 (1971).
8. V. V. Fedorov, Fiziol. Zh. SSSR, 55, 588 (1969).
9. K. W. Engel and R. L. Irwin, Am. J. Physiol., 213, 511 (1967).
10. M. Kuno, S. A. Turkanis, and G. N. Weakly, J. Physiol. (London), 213, 545 (1971).
11. G. Lännergren and R. S. Smith, Acta Physiol. Scand., 68, 263 (1966).

EFFECT OF FEEDING ON ELECTRICAL ACTIVITY OF THE DUODENAL SMOOTH MUSCLES IN DOGS

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Electrical activity of the duodenal smooth muscles was studied with the aid of permanently implanted electrodes. This activity was compared in the fasting state and at a time after feeding equal to the duration of the resting period and the period of activity of the duodenum outside digestion. Duodenal activity after feeding, as reflected in the number of pace-setting potentials, corresponded to its fasting activity. Duodenal activity during digestion differed considerably as regards the number of spike volleys outside digestion. The ratio between "digestive" and "fasting" duodenal electrical activity depended on the type of potentials and the periods of time compared. The optimal nature of "digestive" activity of the duodenal smooth muscles is evidently reflected in the fact that this activity fluctuates within certain limits during digestion: between maximal activity during the period of work and minimal activity during the period of rest outside digestion.

KEY WORDS: duodenum; electrical activity before and after feeding.

Electrical activity of the smooth muscles of the gastrointestinal tract takes the form of pace-setting and spike potentials. The pace-setting potentials (slow electrical waves) determine the highest possible frequency of contractions of the smooth muscles. Spike potentials, i.e., action potentials (AP), are connected with the presence of these contractions [6-9]. The ratio between the number of volleys of AP and the number of pace-setting potentials (the percentage of spike activity) is one of the most informative indices of the motor function of the gastrointestinal tract. However, information on the value of this index for the duodenum during its periodic activity and during digestion is limited in amount and contradictory in nature [7-11].

The object of this investigation was to study the effect of feeding on electrical activity of the duodenal smooth muscles in dogs, allowing for periodic fluctuation of this activity outside digestion.

EXPERIMENTAL METHOD

Experiments were carried out on four dogs weighing 20-28 kg. Silver loop electrodes were implanted into the smooth-muscle layer of the middle third of the duodenum (interelectrode distance 5-10 mm). The plug and socket unit for the electrodes was fixed subcutaneously on the anterior abdominal wall. The derived potentials were recorded on encephalograph. Experiments began 10-14 days after implantation of the electrodes. Each experiment started 16-18 h after feeding and was repeated once or twice a week for one or two months. Six experiments were performed on each animal. At least one periodic cycle of duodenal activity was recorded in the fasting state. The animals were fed differently in the course of the ex-

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