

# MICROELECTROPHYSIOLOGICAL ANALYSIS OF THE FIBER COMPOSITION OF THE RAT DIGASTRIC MUSCLE

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The digastric muscle (DM) plays an active part in all types of movements of the lower jaw. It is innervated by the motor nuclei of the V and VII nerves. In response to strong stimulation of the oral mucosa, pulp of the tooth, and certain areas of skin of the face, the defensive reflex of mouth opening arises mainly on account of contraction of DM.

Histochemical investigations have demonstrated the heterogeneity of the fiber composition of the anterior and posterior bellies of DM [4, 6]. Meanwhile, according to clinical observations [2, 3, 5], during prolonged changes in the conditions of motor activity and also in many diseases of the maxillofacial system, the function of the mouth-opening muscles may undergo appreciable disturbances, which are evidently connected with reorganization of the fiber composition. However, investigation of the pathogenetic mechanisms of disturbance of activity of the mouth-opening muscles requires determination of the quotas and properties of different types of muscle fibers, above all in intact experimental animals.

In the investigation described below parameters of different types of fibers constituting the anterior and posterior bellies of DM were determined in albino rats, the most widely used species of experimental animal.

## EXPERIMENTAL METHODS

Experiments were carried out on noninbred albino rats weighing 160-200 g. The animals were anesthetized intraperitoneally with pentobarbital (3-4 mg/100 g body weight). The anterior or posterior belly of DM was dissected and the fascia partly removed. The skin and adjacent tissues were formed into a bath, which was filled with warm mineral oil (37°C). The animal was fixed to a frame for microelectrode investigations, the jaws were fixed with metal clips attached to the upper and lower incisors, and the corresponding muscle belly was stretched with a glass hook by the intermediate tendon. To record resting membrane potentials (RMP) and, at the same time, to stimulate the cells, glass (Pyrex) microelectrodes filled with 3 M potassium chloride solution (resistance 4-10 MΩ) were used. Intracellular testing of the cytoplasmic membrane of the fiber by depolarizing pulses of current was carried out through the same microelectrode, incorporated into a bridge circuit [1]. The critical depolarization level (CDL), the amplitude of the action potential (AP), the latent period (LP), and other parameters were studied. The microelectrodes were inserted into the muscle to a depth of not more than 2.5 mm (the thickness of both bellies was 3-3.5 mm). The experimental results were subjected to statistical analysis.

## EXPERIMENTAL RESULTS

The initial aim was to determine RMP of the fibers in the composition of the anterior and posterior bellies of DM. Figure 1a shows that compared with the posterior belly, the anterior contained fewer fibers with low polarization levels, and consisted mainly of myocytes with RMP of 70-90 mV. The posterior belly was found to contain many myocytes with RMP of 50-70 mV (Fig. 1b).

The next step was to study whether RMP differ in muscle fibers located at different depths from the muscle surface. The experimental results showed that the laminar organization

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TABLE 1. Electrophysiological Characteristics of Fibers of Rat DM

Layers of bellies of DM	Statistical index	AP, mV	Rheobase stimulating current, nA	CDL, mV	LP, msec	Negative after-potential	
						depth, mV	duration, msec
Anterior belly							
I outer	$M \pm m$ $n$	76,0 $\pm$ 2,3 22	9,57 $\pm$ 0,93 34	12,08 $\pm$ 0,47 22	6,00 $\pm$ 0,31 32	6,43 $\pm$ 0,57 18	6,15 $\pm$ 0,33 33
II inner	$M \pm m$ $n$	81,0 $\pm$ 2,8 13	8,05 $\pm$ 1,10 15	12,28 $\pm$ 0,57 12	6,97 $\pm$ 0,50 16	5,63 $\pm$ 0,46 9	5,97 $\pm$ 0,44 17
Posterior belly							
I outer	$M \pm m$ $n$ $P$	79,4 $\pm$ 1,8 48	8,48 $\pm$ 0,60 45	14,18 $\pm$ 0,36 40 0,001	4,92 $\pm$ 0,24 49 0,01	8,29 $\pm$ 0,40 36	6,57 $\pm$ 0,29 48
II inner	$M \pm m$ $n$ $P$	68,6 $\pm$ 2,5 15 0,01*	8,92 $\pm$ 1,39 13	13,97 $\pm$ 0,56 12 0,05	4,32 $\pm$ 0,22 14 0,001	7,00 $\pm$ 0,45 13	6,64 $\pm$ 0,39 14

Legend. \*) P calculated in the same way also compared with the outer layer of the posterior belly.

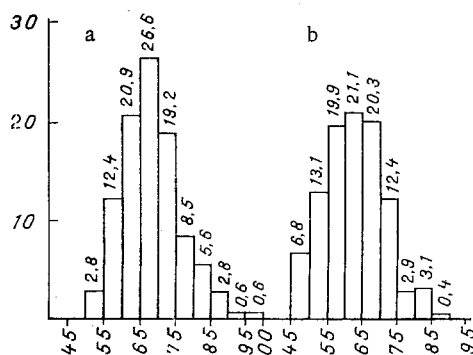


Fig. 1

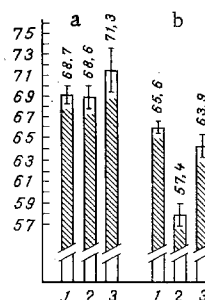


Fig. 2

Fig. 1. Histograms of distribution of RMP of rat DM fibers: a) anterior belly ( $n = 300$ ); b) posterior belly ( $n = 255$ ). Abscissa, RMP of muscle fibers (in mV); ordinate, number of fibers (in %).

Fig. 2. Distribution of RMP of rat DM fibers depending on depth in the muscle: a) anterior belly ( $n = 300$ ); b) posterior belly ( $n = 255$ ). Abscissa, layers of muscle fibers: 1) 0.001-1.00 mm, 2) 1.01-1.70 mm, 3) 1.71-2.50 mm; ordinate, RMP (in mV).

of the anterior and posterior bellies of DM differs. It will be clear from Fig. 2 that the muscle fibers of the anterior belly had approximately the same mean value of RMP in all layers. This is evident that fibers with high, average, and low levels of polarization are distributed uniformly throughout the depth of the anterior belly. In the posterior belly, on the other hand, the mean value of RMP of the myocytes in the middle layer was much less than that in the surface and deep layers, where the mean values of RMP of the myocytes were about equal. Taking this into consideration, and also the fact that the posterior belly of DM is spindle-shaped, it can be concluded that a high proportion of fibers with low and average levels of polarization in the posterior belly of DM is located in the core, whereas myocytes with high RMP predominate in the outer layer.

On the basis of data showing a difference between parameters of excitability of the cytoplasmic membrane of the different types of muscle fibers [7, 8], it was decided to determine the ability of fibers of the anterior and posterior bellies of the rat DM with high, average, and low polarization levels to generate high-voltage spikes in response to depolar-

ization of their membrane through a microelectrode inserted into the cell. The investigations were carried out separately on the outer and inner layers of the two bellies of DM. It will be clear from Table 1 that not only the values of RMP, but also CDL and LP (outer and inner layers) and the amplitudes of AP (inner layer) differ in the anterior and posterior bellies. Comparison of the parameters of excitation of the myocytes in the outer and inner layers of the posterior belly of DM revealed a difference only in the amplitude of the AP generated. All these facts taken together indicate that there are unequal quotas of muscle fibers of different types in the anterior and posterior bellies of DM. More excitable fibers with high levels of polarization predominate in the anterior belly, whereas less excitable fibers with low and average levels of polarization predominate in the posterior belly. Incidentally, the results of electrophysiological testing of the types of muscle fibers agree largely with the results of histochemical investigations of the fiber composition of DM [4, 6].

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#### EFFECT OF COMPLEMENT COMPONENT Clq ON BLOOD COAGULATION AND FIBRINOLYSIS

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Complement plays its primary role in the regulation of and interconnection between the cellular and humoral components of immune reactions and, consequently, in all physiological functions of the body including the hemostasis system, rather than in the efferent system of protection of the organism [2]. Its highly active substances include activation products of the complementary cascade C5a, C3a, C4a, C3b, etc., that under normal conditions are quickly inactivated. Meanwhile physiologically active components may also be found in the form of inert complexes, whose biological effect is induced during their formation and dissociation. Accordingly, the study of the subcomponents of the first component of complement and, in particular, of Clq, is of great interest.

This paper describes a study of the effect of Clq on blood coagulation and fibrinolysis *in vitro*, undertaken for the first time.

#### EXPERIMENTAL METHODS

Clq was isolated by affinity chromatography [3] on macroporous glass from the euglobulin fraction of serum. The preparation contained IgG and fibronectin. The investigation was conducted on whole blood and platelet-enriched and platelet-depleted plasma from healthy blood donors, and plasma deficient in factors V, X + VII. To 0.1 ml of plasma an equal quantity of Clq was added in concentrations of 240, 120, 60, 30, 15, 8, 4, and 2  $\mu\text{g/ml}$ , which was followed by incubation for 30 sec and addition of an agent to initiate clotting. In the con-

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