A Study of Excitation-Contraction Coupling in Frog Tonic Muscle Fibers of *Rana temporaria*

There is every evidence to suggest that the activation of contraction in frog tonic (slow) muscle fibres has some peculiarities[1-6]. Besides, the electron-microscope studies have shown that the T-system in these fibres is less developed than in phasic ones[7,8].

It is well known that glycerol removal from phasic muscle leads to dissociation of excitation-contraction coupling (ECC) caused by disconnection of the tubules membrane elements from the surface membrane[9-12]. It appears that the removal of 400 m*M* glycerol from the tonic bundle of frog's m. ileofibularis, containing phasic and tonic fibres, leads to the disappearance of phasic contraction, but does not affect tonic contraction[13]. These results suggest that in tonic muscle fibres the disconnection of T-system from the surface membrane does not cause dissociation of ECC, and consequently, this system does not play any significant role in spreading excitation inside tonic fibres. For these reasons tonic muscle fibres were referred to the type of fibres with a 'directly coupling system', i.e. to fibres in which ECC is induced directly by the surface membrane[14].

To detect peculiarities of ECC we studied the glycerol effect on isolated tonic fibres of m. ileofibularis in *Rana temporaria*. Tonic fibres were identified by their capacity of maintaining potassium contraction[1,2].

The exposure of tonic muscle fibres to a Ringer containing 400 m*M* glycerol (R + 400G) induced contraction of about the same amplitude as the maximum potassium contracture (Figure 1). A few minutes after, the contraction broke off. After 1 h of exposure in R + 400G, the fibre was returned to the Ringer, and the normal potassium contraction was recorded. In 15 min the contraction capacity vanished. There was no response 2 h later. The intracellular microelectrode recording showed that fibres retained membrane potential and responded to the increase of potassium ion concentration by depolarization. At the same time normal caffeine contracture could be recorded (Figure 1). Hence it can be concluded that the lack of contraction is not related either to changes in the properties of surface membrane, or to disturbances in the contractile apparatus itself. The same results were obtained from experiments in which

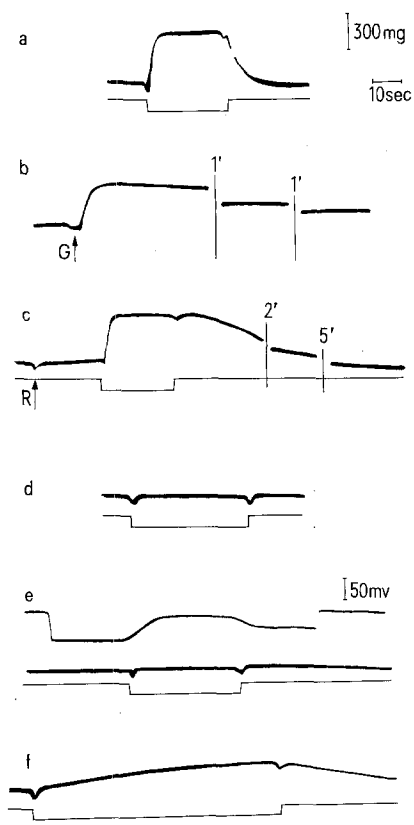

Fig. 1. Glycerol effect on potassium contraction of isolated frog tonic muscle fibre.

a) normal potassium contraction; b) contraction caused by 400 m*M* glycerol application (R + 400G); c) immediately after glycerol removal; d) 15 min after glycerol removal; e) 2 h after 400 G removal during depolarization of membrane); f) caffeine contraction 2 h after glycerol removal.

In each record: Upper line, isometric fibre contraction as recorded by means of RCA 5734 transducer tube; lower line, indicating increase in potassium ion concentration up to 80 m*M* (at the expense of appropriate decrease in Na ions). f) caffeine application (7 m*M*). The upper line in e) microelectrode recording of membrane potential.

The potassium contraction of phasic and tonic isolated muscle fibres 1 h after glycerol removal in % of the original value

Fibre	Phasic fibres		Fibre	Tonic fibres		
No.	R+50G	R+100G	No.	R+200G	R+400G	R+200G[a] R R+400G
1	79	0	1	80	0	28
2	39	0	2	70	0	30
3	23	0	3	100	0	21
4	54	0	4	113	0	33
5	114	0	5	85	0	40
6	22	0				
Mean	55	0		90	0	30

[a] Sequence of the experimental procedure:
1h R + 200G; 2 h R; 1 h R + 400G; 1 h R.
R, Ringer solution; 50G 100G, 200G, 400G, concentration of glycerol in m*M*.

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contraction was induced by 5×10^{-6} M acetylcholine-chloride solution.

In the case when tonic muscle fibres are kept for 1 h in R + 200G, their contraction capacity after glycerol removal is found to be reduced about twice, and 2 h later is restored completely. If after that the tonic fibres are transferred to R + 400G for 1 h and washed out once more, the contraction retains. As was demonstrated on 5 fibres under investigation the amplitude of contraction in 1 h after the second glycerol removal was 20–40% of the original value (Table). Thus the preliminary removal of glycerol lower concentration reduced the disconnection effect of higher concentration. The same effect was obtained in 6 experiments on phasic fibres.

The ultrastructure of the isolated tonic fibre undergoes considerable changes after 1-hour incubation in R + 400G followed by 2-hour wash out in the Ringer solution. Large vacuoles can be seen between myofibrils in sites of localization of sarcoplasmic reticulum elements. Moreover, small vacuoles occur inside myofibrils. The membranes around these vacuoles could not be seen (Figure 2). In the second experimental variant, when the fibre was kept in R + 200G and then re-incubated in R + 400G, alterations in the ultrastructure were not so strong. There were no small vacuoles inside myofibrils. Large vauoles were rare and, as a rule, retained their membranes.

To understand the difference between our data and the results obtained by STEFANI and STEINBACH[13] we performed experiments on muscle bundles consisting of about 15–20 various fibres. In this case, as in the experiments of the above authors, the phasic contraction component disappeared completely after the removal of 400 mM glycerol, while tonic contraction was retained. It appears that different effects of glycerol removal on isolated fibre and bundle of fibres are caused by variation in the rates of glycerol removal. There is evidence that the low speed of glycerol efflux does not destroy the T-system[12]. Thus it was shown that tonic fibres are more resistant to the deleterious effect of glycerol removal than phasic ones (Table).

As in the case of phasic fibres, the tonic fibre membrane responds to high potassium ion concentration by depolarization without contraction of fibres. Caffeine, nevertheless, induces contraction. This indicates that in both types of the fibres ECC is dissociated. In this context it can be suggested that in tonic fibres, as in phasic ones, vacuoles are formed due to the T-system swelling. Thus it implies the same mechanism of glycerol removal effect on tonic and phasic fibres. Since there is no essential difference in the diameter of tonic and phasic fibres, it is hard to suppose that in the former case activation of contraction is accomplished directly by the surface membrane. The slowness of contraction may be explained by scarce T-system elements as compared with phasic muscle fibres[7,8]. Moreover, the appearance of vacuoles during glycerol removal need not have prevented excitation spreading inside the fibre had it been brought about by direct diffusion of activator from the surface membrane.

Thus the above evidence shows that there is no essential difference between phasic and tonic fibres as regards the mode of spreading excitation from the membrane to contractile apparatus. Quantative difference in the development of the T-system appears to be responsible for higher resistence of tonic fibres to glycerol removal.

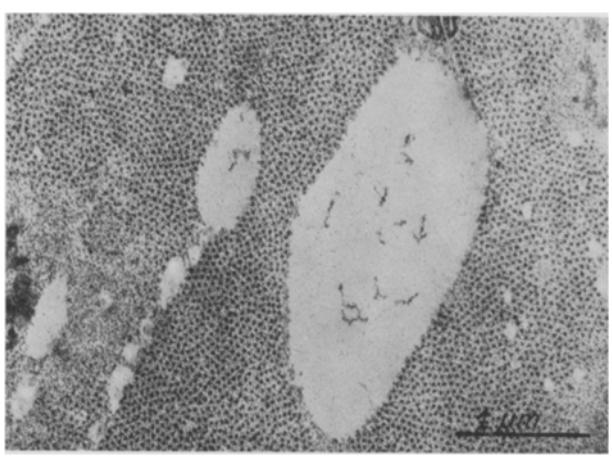

Fig. 2. Changes in the ultrastructure of frog tonic muscle fibres after incubation in glycerol solution (R + 400 mM G) for 1 h and its removal for 2 h. Fixation in 2.5% glutar aldehyde in cacodilate buffer at pH 7.2; Epon 812 embedding. GEM — 7; × 25,000.

ВЫВОДЫ. На основании регистрации сокращений, мембранного потенциала и изучения ультраструктуры изолированных тонических мышечных волокон лягушки при действии глицерина делается заключение о том, что в этих волокнах, также как в фазных, Т-система необходима для осуществления электромеханической связи.

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D- and L-Isomers of Serine and Alanine Equally Effective as Releasers of Gastrin

We[1] recently reported that L-phenylalanine releases cholecystokinin but D-phenylalanine does not when solutions of them are perfused into the intestine. We report here that D- and L-isomers of serine and alanine are equally effective as releasers of gastrin when solutions of them bathe the mucosa of the pyloric gland area of the stomach. The studies were done on 3 dogs with vagally innervated pouches of the pyloric gland area (antrum) and vagally innervated pouches of the oxygenic gland area (Pavlov type). Solutions to be tested were introduced into the antral pouch and the effect on rate of secretion of acid from the oxyntic gland pouch was measured. The amino acids tested, glycine, alanine, and serine, are known to be among the most effective ones in releasing gastrin[2], and in the present tests the responses to these 3 amino acids did not differ significantly (Table). We found no previous reports comparing D- and L-isomers of amino

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