THE ROLE OF THE ACETYLCHOLINE – CHOLINESTERASE SYSTEM IN THE ORIGIN OF BIOELECTRICAL PHENOMENA IN SKELETAL MUSCLE

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In 1938 A. G. Ginetsinskii and N. I. Mikhel'son [1] put forward the view that a connection existed between the state of the acetylcholine in a muscle and the bioelectrical phenomena. These authors found that direct stimulation of a muscle poisoned with eserine led in addition to the development of a contracture, to an increasing negative electrical potential. The action currents gradually diminished and reached negligible values.

They came to the conclusion that "the widespread idea of a single mechanism lying at the basis of the production of the injury potential and the action potential is illustrated very expressively by the electrical phenomena in a muscle poisoned with eserine, which may be regarded as something occupying a middle position between the action current and the demarcation current".

In the same year Kh. S. Koshtoyants put forward the hypothesis of a connection between the synthesis of acetylcholine and the carbohydrate-phosphorus metabolism, which was further developed in a series of subsequent investigations [4, 5, 6, 7, and others]. On the basis of these findings, Kh. S. Koshtoyants came to the conclusion that the bioelectrical phenomena were connected in their origin with the state of the carbohydrate-phosphorus metabolism and with the state of the acetylcholine – cholinesterase system [5, 6, 7 and others].

During the period 1941-1947, Nachmansohn and his co-workers developed the theory of the relationship between the action currents of different tissues (nerve muscle, the electric organ of fishes) and the state of the acetylcholine–cholinesterase system.

From a summing up of the experimental data, Nachmansohn and his co-workers [11] reported that the view of the role of acetylcholine in the origin of the bioelectrical phenomena was based: 1) on the presence of a high concentration of cholinesterase in nerves, which shows that large amounts of acetylcholine must be hydrolyzed in the course of a millisecond; 2) on findings that cholinesterase is localized in the superficial layer of the nerve; and 3) on the close parallel between the concentration of cholinesterase and the maximum emf of the action current. By direct measurements [12] they showed the proportional relationship between the cholinesterase activity and the magnitude of the discharge of the electric organ.

These results were confirmed later both by experiments in which nerve and muscle were poisoned with eserine and strychnine, paralyzing the cholinesterase [8, 9] and by experiments with DFP (di-isopropylfluorophosphate), which also inactivates cholinesterase [9].

These authors showed that strychnine in a concentration of 0.0005 and 0.001 M and eserine in concentrations of 0.01, 0.005 and 0.02 M lead to disappearance of the action currents of nerve and muscle. This was explained by the fact that strychnine and eserine are inhibitors of cholinesterase, with the result that after the administration of these drugs, the acetylcholine secreted at the moment of excitation gives a persistently negative potential to the membranes, for it does not undergo hydrolysis for a long time.

These hypotheses, however, aroused a number of objections both in relation to the DFP experiments and in relation to experiments with eserine, strychnine and proserine [2, 10].

S. D. Kovtun [3] came to the conclusion that acetylcholine cannot be the agent which directly stimulates the process of excitation in nerves and muscles, for during its action on a nerve, electrical motor phenomena are not generally observed, and during its action on muscle, the negative potential reached only 10-20 mv, whereas during the process of excitation the negative potential reached 35-60 mv.

Certain differences of opinion thus exist in our understanding of the role of the acetylcholine—cholinesterase system in the generation of action currents, and there are discrepancies in the experimental findings on this subject.

Our experiments were undertaken in order to study the role of the acetylcholine—cholinesterase system in the origin of bioelectrical phenomena.

TABLE 1

The Effect of Strychnine ($1:10^4$) and Eserine ($1:10^4$) on the emf of Injury of the Skeletal Muscle of the Frog (mean of 5 experiments)

Time from beginning of expt. (in hours)	emf in % of initial value during the action of				
	control	on the injured portion of the muscle		on the uninjured portion of the muscle	
		eserine	strychnine	eserine	strychnine
1	80	84	81	86	83
2	74	76	71	80	75
3	68	74	66	78	67
4	65	67	64	72	65
5	62	65	62	71	63
6	59	59	57	62	58

TABLE 2

The Effect of Strychnine ($1:10^3$) and Eserine ($1:10^3$) on the emf of Injury of the Skeletal Muscle of the Frog (mean of 5 experiments)

Time from beginning of expt. (in hours)	emf in % of initial value during the action of				
	control	on the injured portion of the muscle		on the uninjured portion of the muscle	
		eserine	strychnine	eserine	strychnine
1	86	90	80	82	81
2	81	82	80	76	57
3	76	77	73	75	52
4	73	72	71	72	38
5	72	68	66	70	37
6	63	64	66	67	31

EXPERIMENTAL METHOD

Experiments were carried out on the isolated sartorius muscles of autumn and winter frogs. We measured the rate of fall of the electromotive force (emf) of injury and the changes in the value of the emf of excitation under the influence of the action of solutions of eserine in concentrations of $1:10^4$ and $1:10^5$ and of strychnine, $1:10^3$, $1:10^4$ and $1:10^5$.

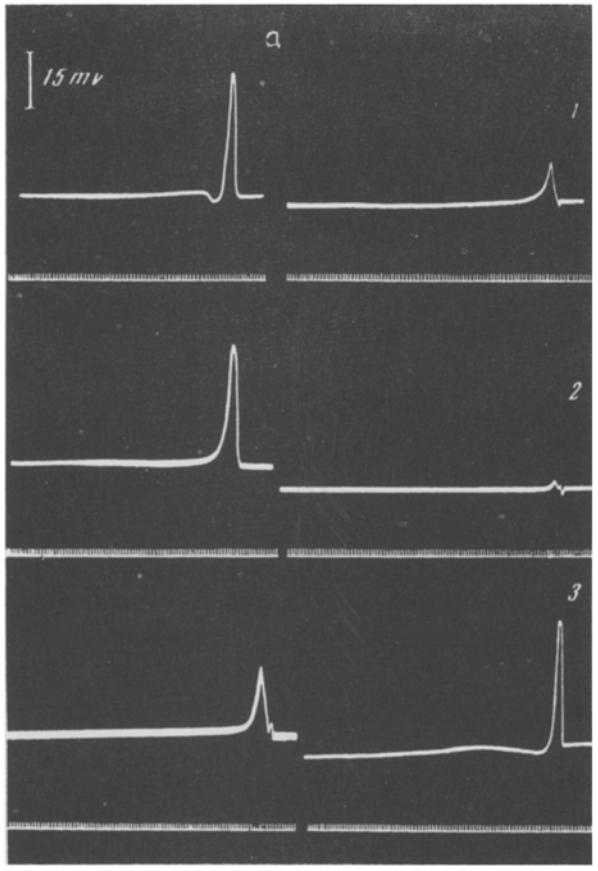

Fig. 1. The effect of poisoning an isolated muscle with strychnine ($1:10^4$) on the emf of excitation. A: 1) Initial tracing; 2) after a stay of 15 minutes in the solution; 3) after 30 minutes. B: 1) after a stay of 45 minutes in the same solution; 2) after 60 minutes; 3) the opposite muscle after a stay of 60 minutes in Ringer's solution. Time marker 2 milliseconds.

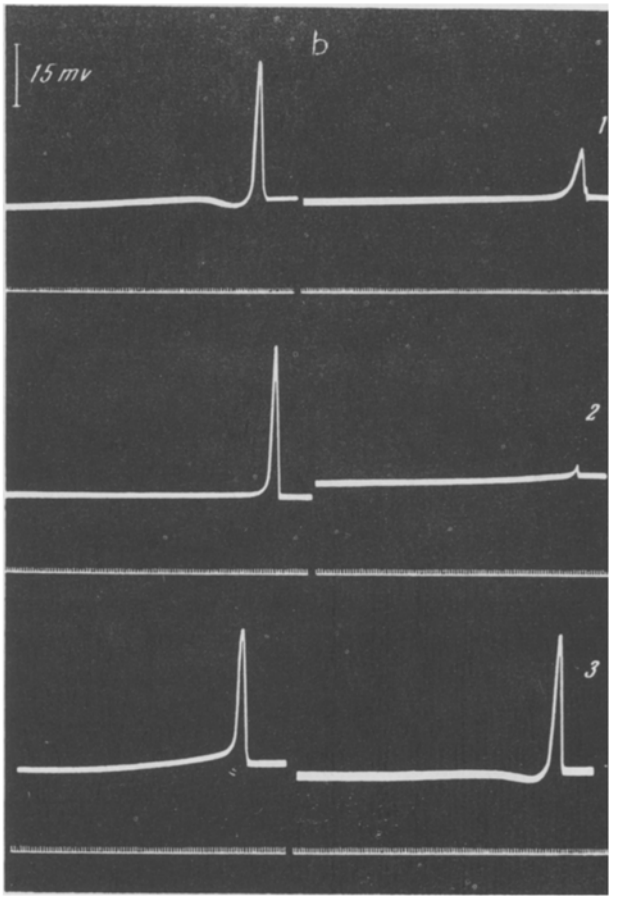

Fig. 2. The effect of poisoning an isolated muscle with eserine ($1:10^4$) on the emf of excitation. Legend as in Fig. 1.

In order to measure the emf of injury, the pelvic end of the sartorius muscle was crushed by means of a special clamp and the muscle was placed in a paraffin wax bath so that its injured end was in one compartment and the uninjured end in the other. Between these compartments a partition was created from a mixture of paraffin wax and paraffin oil. Into each compartment of the bath, Ringer's solution or one of the test solutions was poured at the time of the experiment.

The emf of injury was measured by a compensation method, using ordinary ($Zn-ZnSO_4$) nonpolarizing electrodes with agar taps. In all,40 such experiments were performed.

During the recording of the emf of excitation the muscle was kept on a wax block in a humid chamber. To avoid distortion of the tracing by contraction, the muscle was fixed in a slightly stretched position.

The excitation current was led from the injured and uninjured areas of the muscle by means of nonpolarizing electrodes to a loop oscillograph (loop T-5). When such leads were used the current was always monophasic.

Direct stimulation of the muscle was carried out by impulses of current above threshold value, with a duration of 10 milliseconds and at intervals of 1 second. The distance between the platinum stimulating electrodes was 1-1.5 mm, the distance between the stimulating and the lead electrodes was 10 mm, and that between the lead electrodes 5-10 mm.

In all,20 experiments were carried out, in 10 of which the muscle was poisoned with strychnine and in 10 with eserine.

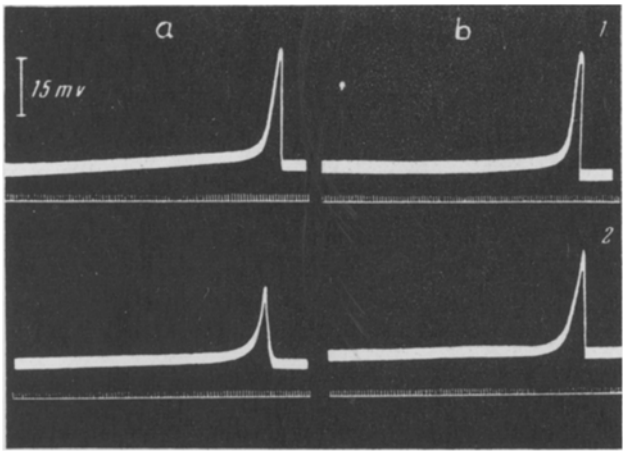

Fig. 3. The effect of poisoning an isolated muscle with eserine – 1 ($1:10^5$), with strychnine – 2 ($1:10^5$) on the emf of excitation. A) After a stay of 60 minutes in the solution; B) initial state. Time marker 2 milliseconds.

EXPERIMENTAL RESULTS

In Table 1 are shown the results of experiments with changes in the emf of injury, showing that strychnine and eserine, in dilutions lower than those used by Nachmansohn, had no appreciable effect on the emf of injury, except in cases where strychnine acted on the uninjured segment of the muscle.

In Figs. 1 and 2 are shown the results of experiments on the effect of eserine and strychnine on the value of the emf of excitation, from which it can be seen that eserine and strychnine in concentrations of $1:10^4$ caused obvious changes in this emf. Under these circumstances strychnine produced more considerable changes in the magnitude and duration of the peak of the emf of excitation.

Fig. 3 illustrates the changes in the emf of excitation during poisoning of the muscle with $1:10^5$ solutions of strychnine and eserine. As is seen from Fig. 3, strychnine, in this concentration, causes comparatively small changes in the emf of excitation, while eserine produces practically no change.

The experiments described do not support the conclusions of A. G. Ginetsinskii and N. I. Mikhel'son that the mechanism of origin ofthe injury and excitation currents in skeletal muscle is the same.

According to our findings, strychnine and eserine are capable of producing changes in the magnitude and duration of the current of excitation, but have practically no effect on the magnitude nor the rate of fall of the injury current, as is particularly clearly seen after poisoning the muscle with eserine.

From the findings described above, certain differences may thus be discerned in the mechanism of origin of the currents of the injury and excitation in skeletal muscle, at least in respect to the participation of the acetylcholine—cholinesterase system in this process.

SUMMARY

Poisoning of the isolated sartorius muscle in frog with eserine and strychnine in the $1:10^5$ and $1:10^4$ concentrations causes the change of the value and the form of the excitation biocurrent. Strychnine and eserine in the $1:10^5$, $1:10^4$ and $1:10^3$ concentrations have no effect on the electromotive force of the sartorius muscle injury. These data lead us to presume certain differences in the mechanism of the origin of the excitation and injury biocurrents in the skeletal muscle, at least with relation to the participation of the acetylcholine—cholinesterase system in this process.

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