# THE ROLE OF ACETYLCHOLINE IN THE DEVELOPMENT OF TETANIC SINGLE RESPONSE AND AFTERPOTENTIALS IN THE NERVE TRUNK OF COLD-BLOODED ANIMALS

G. I. Poletaev

Department of Normal Physiology (Head – Doctor of Medical Sciences, I. N. Volkova),

Kazan Medical Institute (Head – Assistant Professor R. A. Vlaselev)

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The phenomenon of tetanic single contraction described by N. E. Vvendenskii in 1886 [4] is of great theoretical importance since it is a peripheral model of the processes of summation of excitation which occur in the central nervous system. The phenomenon of tetanic single contraction consists of a single wave of excitation, passing through an area of sub-threshold tetany, giving rise to muscle contraction which acquires tetanic character. Academician A. A. Ukhtomskii [14] saw in the mechanism of the formation of a tetanic single contraction such relationships as could take place in the formation of a dominant. A number of authors [1, 10, 11] have shown that the passing wave of excitation activates local processes in the nerve trunk elicited by sub-threshold tetanization. When recording action potentials from the nerve trunk or fiber it is possible to speak not of tetanic single contraction but tetanic single response [11]. A depressed functional state of the nerve trunk forms a favorable condition for the formation of the tetanic single response [4, 10, 13].

The cause underlying the appearance of tetanic single response is considered to be the low voltage negative afterpotential, especially since a connection has been established between the latter and the phase of increased excitability [3, 6, 10, 13, 14, 16]. Many investigators consider that metabolic processes underly the afterpotentials [5, 15]. All the factors which enhance the negative afterpotential lead to improved conditions for demonstration of tetanic single response, and vice versa [3, 13, 14, 17].

The present work is concerned with investigation of the role played by the mediator acetylcholine in the development of negative afterpotential and the phenomenon of tetanic single response, since this aspect has so far received little attention [2, 8].

## EXPERIMENTAL METHOD

Experiments were performed on a preparation of n. ischiadicus-n. peroneus of frog (Rana ridibunda). The nerve trunk was placed on 3 pairs of electrodes: maximal stimulation was applied at the proximal electrodes, sub-threshold or threshold tetanization was effected at the middle electrodes, and the distal electrodes were chlorided and used for recording. A grounded silver plate was placed between the middle and distal pairs of electrodes in order to isolate the path of stimulating current. During the experiment the nerve trunk was placed into a specially constructed humid chamber. Two electronic stimulators were used as stimuli. The action potentials were recorded on a two-channel electron oscillograph. Since very low frequencies could be reproduced, it was possible to record almost without distortion the slow afterpotentials in the nerve trunk. During investigation of afterpotentials only 2 pairs of electrodes were used, stimulating and recording. The latter had an interelectrode distance of 2 cm. In order to avoid dispersion in time of the excitation waves in the nerve trunk the distance between the two pairs of electrodes was under 1 cm. To obtain monophasic action-potential waves the peripheral end of the nerve was crushed or treated by heat. The role of acetylcholine was studied by removal of the pancreas, a procedure which, according to a number of investigations [7, 9, 12] impairs the formation of acetylcholine in the organism. The operation for removal of the pancreas was carried out under sterile conditions 4-10 days prior to the experiment. The effect of acetylcholine and eserine was studied by introducing these agents in various concentrations into the posterior lymph sac of frogs. The experiments were performed on winter-spring frogs. A total of 167 experiments was carried out.

## EXPERIMENTAL RESULTS

It was possible to obtain tetanic single response fairly frequently at the very beginning of experiment on the nerve trunks of normal frogs when the frequency of sub-threshold stimulation was 50 per second. Such tetanic single response consists of a whole series of action potentials following the frequency of the sub-threshold stimulation quite synchronously and appearing after the action potential elicited by a single stimulus of maximal strength. The amplitude of the action potentials constituting the tetanic single response decreases gradually until complete disappearance. The total duration of the tetanic single response obtained at the very beginning of an experiment is 0.34 sec* (Fig. 1,a). Tetanic single response was never observed at the beginning of an experiment when the frequency of the sub-threshold or threshold stimulation was 25 per second (Fig. 1,b).

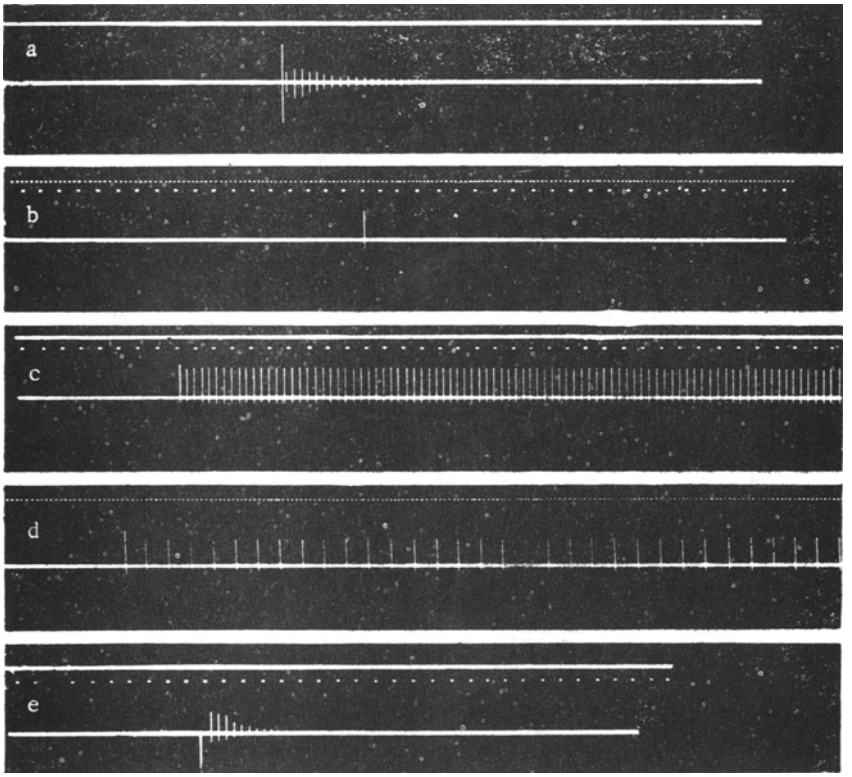


Fig. 1. Electrograms of tetanic single response. a, b) Tetanic single response during 5th min of experiment (frequency of sub-threshold stimulation 50 and 25 per second); c, d) the same on 7th day after removal of pancreas (frequency of stimulation 50 and 25 per second); e) the same on the 7th day after operation with compensatory administration of acetylcholine (frequency of stimulation 50 per second). Records from above down: time marker (0.01 and 0.05 sec), electrogram.

In the case of the nerve trunks of frogs whose pancreas had been removed, tetanic single response could be obtained in all animals without exception on the 6-10th postoperative day; it should be mentioned that from the very first minutes of the experiment the response acquired a "prolonged" character, i. e., the action potentials of the tetanic single response did not show a tendency to disappear rapidly but persisted sometimes up to 30-60 sec with maintenance of quite a high amplitude (Fig. 1,c). Such results were obtained not only with sub-threshold stimulation at a frequency of 50 per second but also at 25 per second (Fig. 1,d). This sort of "prolonged" type of tetanic single response could only be obtained on nerve trunks of normal animals after preliminary supra-threshold stimulation lasting 10-20 min at frequencies of 50-100 per second.

* Average figures are cited in this paper.

Nerve trunks of operated animals who had been systematically given compensatory doses of acetylcholine (1:10,000 solution, 0.5 ml daily from the 3rd-4th postoperative day introduced into the posterior lymph sac) showed tetanic single response at the beginning of experiment which was never "prolonged" (Fig. 1,e); in many cases it was absent altogether.

Introduction of eserine in 1:1000 concentration (1 ml) into the posterior lymph sac of normal animals 40-50 min prior to the experiment caused a sharp deterioration in the conditions for demonstration of tetanic single response; however, eserine in concentration 1:10,000 (1 ml) exerted no significant effect on the course of tetanic single response.

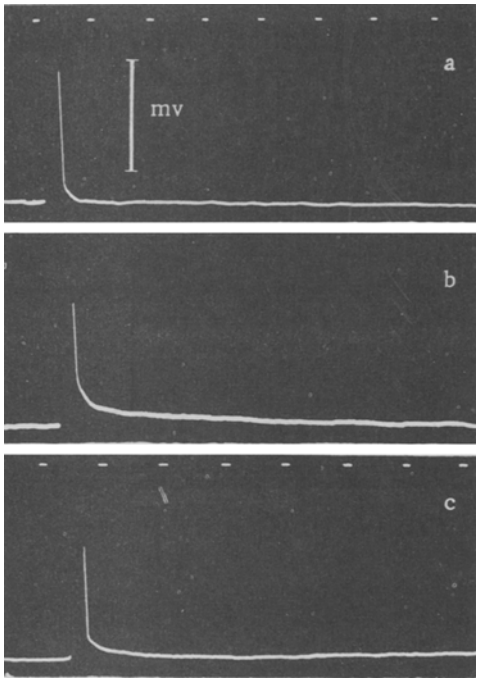


Fig. 2. Electrograms of nerve action potentials. a) Negative afterpotential in nerve of normal frog; b) negative afterpotential on the 7th day after removal of pancreas; c) negative afterpotential of nerve trunk with acetylcholine compensation. The summits of the action potential spikes pass beyond the limits of the records and in all cases reach approximately 4 mv. All the photographs are taken with the same amplification. Calibration 1 mv. Records (from above down): time marker (0.01 sec), electrogram.

Investigation of the negative afterpotential showed that it constituted an average of 4.2% with respect to the spike in the nerve trunks of normal animals; its duration was 0.024 sec (Fig. 2,a). No appreciable positive afterpotential was observed.

Preliminary removal of the pancreas led to marked increase in the negative afterpotential which, under these conditions, constituted 10.27% with respect to the action potential spike and reached 0.1 sec in duration (Fig. 2,b).

Compensatory introduction of acetylcholine to the operated animals from the 3rd-4th postoperative day onwards diminished the negative afterpotential, whose amplitude and duration approached the values obtained from the nerve trunks of normal animals (Fig. 2,c).

Eserine, in concentration 1:1000, administered 40-50 min before the experiment to a normal frog (1 ml) also caused diminution of the negative afterpotential, whose amplitude under these conditions constituted an average of 1.85% with respect to the spike and had a duration of 0.01 sec. Smaller concentrations of eserine (1:10,000, 1 ml) led to slight changes in the negative afterpotential.

Analysis of the experimental results described suggests that the extent to which the negative afterpotential is pronounced depends on the liberation of the mediator acetylcholine.

Studies carried out by A. V. Kibiakov and A. A. Uzbekov have shown that removal of most of the pancreas in frogs causes fatty infiltration of the liver and impairment of acetylcholine synthesis, without significant impairment of sugar metabolism [9]; the impairment of acetylcholine synthesis evidently results from disturbances of fat-lipoid metabolism [12]. According to the data of L. N. Zefirov and A. V. Kibiakov [7], impairment of acetylcholine synthesis leads to lowering of lability, decrease of the resistance of nerve to the action of change-inducing factors.

In our experiments on nerve trunks of depancreatized frogs it was observed that the negative afterpotential was considerably more pronounced, which evidently results from impairment of acetylcholine formation. The presence of quite marked negative afterpotential offers an explanation of the "prolonged" character of the tetanic single response in operated frogs. In this case each wave of excitation of the tetanic single contraction apparently exerts a stronger enhancing effect on the series of following waves which leads to the marked lengthening, "prolongation" in time, of the tetanic single response.

In control experiments in which the operated animals were given acetylcholine the duration of the negative afterpotential decreased and there was concurrent deterioration in conditions with respect to demonstration of tetanic single response.

Our conclusion concerning the significance of acetylcholine in the development of afterpotentials is also confirmed by experiments with eserine. By protecting acetylcholine from rapid hydrolysis, eserine favors diminution of residual processes which, in its turn, determines the difficulty of obtaining tetanic single response in the initial period of an experiment.

The experimental data presented allow the conclusion that acetylcholine favors the diminution of residual processes of excitation in nerve, apparently by exerting a trophic effect.

## SUMMARY

Experiments were performed on the nerve trunks of frogs in which the production of acetylcholine was disturbed by removal of the pancreas. This brought about an increase of the negative afterpotential and prolongation of tetanized single response on the 4th to 10th day following the operation. The compensatory administration of acetylcholine removed these changes in the majority of cases. Preliminary administration of eserine to normal frogs resulted in decrease of the negative afterpotential. With administration of eserine the conditions for appearance of the tetanized single response deteriorated. Evidently, acetylcholine promotes the decrease of the duration of residual processes of excitation in the nerve tissue.

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** Original Russian pagination. See C. B. translation.