VERY WEAK FLUORESCENCE OF FROG NERVE-MUSCLE PREPARATION

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UDC 612.816-085.23:535.37

Very weak fluorescence in the visible region of the spectrum was found in a nerve-muscle preparation of Rana ridibunda at rest and during irrigation of the nerve with acetylcholine solutions (1:1,000,000, 1:1,000, 1:25). The intensity of the very weak fluorescence of the preparation increased with an increase in concentration of acetylcholine. After rinsing the preparation in solutions of ATP or glucose, the intensity of fluorescence rose sharply, but after rinsing in solution of α-dinitrophenol or sodium fluoride it was reduced almost to the background level of the apparatus.

Very weak spontaneous fluorescence, emitted by all living cells, was discovered in animals in 1961 [6]. It is a source of valuable information concerning many processes taking place in the living organism [2, 7].

The object of this investigation was to study very weak fluorescence of a nerve-muscle preparation at rest and during the action of solutions of acetylcholine when phosphorylation and glycolysis were either activated or inhibited.

EXPERIMENTAL METHOD AND RESULTS

Experiments were carried out on isolated nerve-muscle preparations of Rana ridibunda.

Very weak fluorescence of the preparation was detected by means of a special apparatus for the discovery of very weak light fluxes, in which the receiver of the emissions was type FÉU-37 photoelectronic multiplier. Recordings were made by means of an M-21 galvanometer.

Pharmacological acetylcholine chloride was used and was made up when required in physiological saline for cold-blooded animals in concentrations of 1:1,000,000, 1:1,000, and 1:25. The proximal portion of the nerve in the nerve-muscle preparation was irrigated with these solutions.

Phosphorylation and glycolysis were activated by rinsing the preparation for 15 min in a solution (1:500) of a pharmacological preparation of sodium adenosinetriphosphate and a solution (1:100) of glucose, respectively. To inhibit phosphorylation and glycolysis, the preparation was washed for 15 min in 1:500 solution of α-dinitrophenol and 1:25 solution of sodium fluoride, respectively [5].

The nerve-muscle preparation was placed 7 mm from the photocathode of the FÉU-37 instrument. The results were analyzed by statistical methods [3].

Very weak fluorescence of the nerve-muscle preparation at rest was found only in the case of freshly caught frogs in summer. This fluorescence was very weak (from 10.2 ± 0.13 to 11.1 ± 0.10 units), compared with the background for the instrument of 10 units on the scale of a type M-21 galvanometer. The back-

Department of Physiology of Man and Animals, Kazan' University. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 70, No. 8, pp. 48-50, August, 1970. Original article submitted March 11, 1968.

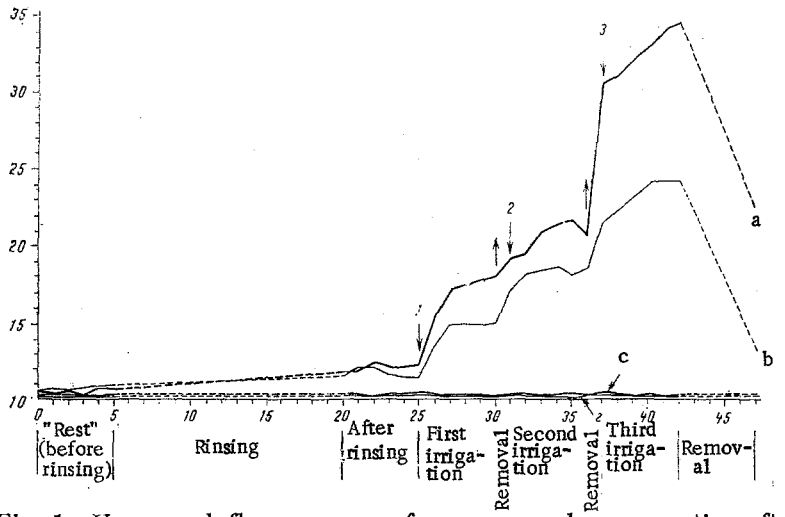

Fig. 1. Very weak fluorescence of nerve-muscle preparation after rinsing in various solutions: a) ATP (1 :500); b) glucose (1 : 100); c) α-dinitrophenol (1 :500); d) sodium fluoride (1 : 25). Arrow pointing downward denotes irrigation of nerve with various concentrations of acetylcholine solution: first irrigation (10^{-6}), second irrigation (10^{-3}), third (1 : 25); arrow pointing upward denotes removal of solutions. Abscissa, time (in min); ordinate, intensity of fluorescence (in conventional units).

ground of the instrument was obtained by counting along the scale of the galvanometer when the instrument was switched on but without the nerve-muscle preparation. A scale reading of 10 units was obtained constantly by means of a special correcting device.

No fluorescence was found in preparations at rest obtained from winter frogs, evidently because of the low resolving power of the instrument, but more especially because of seasonal fluctuations in the frogs [4].

Irrigation of the nerve of the preparation with 10^{-6} acetylcholine solution led to an increase in the intensity of the very weak fluorescence of the nerve-muscle preparation after 10-45 sec to 12.6 ± 0.2 units. Washing out the acetylcholine three times with physiological saline reduced the intensity of the fluorescence to 10.8 ± 0.13 units. The second and third irrigations of the nerve with solutions of the same concentration of acetylcholine (every time after three rinsings with physiological saline) gave a reduced effect of increase in intensity of fluorescence to 11.5 ± 0.16 and 11.4 ± 0.16 units, respectively.

Irrigations of the nerve in the nerve-muscle preparation with acetylcholine solution (10^{-3}) caused an increase in the intensity of fluorescence to 13.2 ± 0.23-14.3 ± 0.12 (after the first irrigation) and to 12.9 ± 0.1 and 12.0 ± 0.15 units, respectively, after the second and third irrigations. If, however, different concentrations of acetylcholine solutions were used to irrigate the same preparation, the intensity of very weak fluorescence increased with an increase in the concentration of the solution.

Preliminary rinsing of the preparation with a solution of sodium adenosinetriphosphate increased the intensity of the very weak fluorescence to 12.3 ± 0.21 units (Fig. 1a). However, if the nerve of such a preparation was treated with acetylcholine solutions, after a latent period of 1-5 min a sharp rise in the intensity of the very weak fluorescence was observed, to 18.0 ± 0.21 after the first irrigation, to 21.6 ± 0.22 after the second, and to 34.5 ± 0.23 units after the third irrigation. Every preceding solution was removed. More concentrated solutions of acetylcholine gave a greater increase in the intensity of fluorescence. The increased intensity of fluorescence was maintained for several minutes, sometimes to 1 h or more. These results are in agreement with data showing that ATP exerts a sensitizing effect on muscle relative to acetylcholine [1].

A similar, but less clearly defined picture was given by preliminary rinsing of the preparation in glucose solution. The intensity of fluorescence, for example, after the third irrigation was increased only to 24.2 ± 0.26 units (Fig. 1 b).

Conversely, after rinsing in a solution of α-dinitrophenol or of sodium fluoride, the intensity of the initial fluorescence of the nerve-muscle preparation was reduced almost to the background level of the instrument. Its value was 10.1 ± 0.1 and 10.1 ± 0.11 units, respectively (Fig. 1 c, d). Subsequent treatment of the nerve of such preparations with acetylcholine solutions as a rule did not cause an increase in the intensity of the very weak fluorescence of the preparation. The value measured did not exceed 10.4 ± 0.16 unit.

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