EFFECTS OF METHYLENE BLUE, ACRIDINE ORANGE, AND ZINC ON MUSCULAR CONTRACTION

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Received June 6, 1960

In this preliminary note we report results of certain studies that developed out of our interest in the action of photodynamic dyes on living skeletal muscle. Previous work in this field (e.g., Lippay (1929); Lillie, Hinrichs and Kosman (1935)) was concerned with effects of non-penetrating, photodynamic acid dyes. Such research deserves further development. But our immediate plan was to study the effects of some penetrating basic dyes. Methylene blue (MB) was of special interest since Eidus and Kondakova (1958) had shown that this dye photodynamically depresses the adenosinetriphosphatase activity of myosin. And acridine orange (AO) appeared of interest because Karreman, Mueller, and Szent-Györgyi (1957) had proved that it decreased contractility of glycerol extracted muscle fibers, evidently by engaging in a competitive adsorption with ATP molecules for reactive sites on the contractile actomyosin. As will be seen, our work showed, as might be expected from the foregoing, that both MB and AO exerted suppressive effects on contraction; but AO in addition could cause a remarkable potentiation of the twitch. Now, our AO contained Zn on a mole to mole basis, and Beers (1960) had demonstrated that the presence of such Zn must be taken into account in explaining the actions of the dye. This was our immediate reason to undertake separate studies of effects of Zn; but we

*We appreciate courtesies given the junior author by his chief, Dr. J. Laughlin, Dept. of Biophysics, Sloan-Kettering Institute, which facilitated his participation in this research.

were also motivated by the interest in this metal that arose from our knowledge of its extraordinary functions in biological systems in general, as recently reviewed by Vallee (1959), and in glycerinated-muscle systems in particular, as shown by Edman (1958, 1959a, 1959b, 1959c).

Methods. Sartorius muscles of the frog, Rana pipiens, were excised, equilibrated in Ringer's solution (generally phosphate-buffered; but for the experiments with Zn as $ZnCl_2$, to prevent precipitation of the Zn as phosphate, it was unbuffered or buffered with imidazole), mounted in a lucite chamber, and connected under an initial tension of 2 gm. to an RCA Type 5734 transducer tube for isometric recording of tension output. Maximal twitches and short tetani were evoked by electrical stimulation and recorded oscillographically. In some experiments diphasic action potentials were recorded by conventional external electrode techniques and were visualized, along with the associated contractions, by means of dual-beam cathode-ray oscillography. In the photodynamic tests, the light of a 10 ampere carbon arc lamp was passed through a 500 cc. beaker of water which, besides acting as a heat filter, cylindrically focussed the beam along the long axis of the muscle. The experiments were done at room temperature. Solutions of substances being tested were applied to the muscle in its chamber. At the time any contractile responses were obtained, the solution was withdrawn and the muscle stimulated in moist air.

Photodynamic Action of Methylene Blue. After tests for normal responses, the muscle was soaked for 30 min. in Ringer's containing 6×10^{-3} mg. MB/ml. (This concentration is the same as that used by Eidus and Kondakova (1958).) Even under the influence of ordinary room lighting, this had no effect on the muscle's contractions. The MB-stained muscle was then for about an hour kept in moist air and exposed to the light from the arc. During this treatment the following occurred. (1) The resting tension slowly increased by about 5 gm. (2) Beginning very soon and progressively developing in amount, both the peak twitch and tetanus tensions decreased, with the twitch diminishing relatively more than the tetanus. (3) The time for the tetanus to

reach plateau increased. These changes are determined by the interaction of both dye and light, since the light of the arc lamp alone had no effect. Whether these changes are truly photodynamic, i.e., requiring also the presence of oxygen, has not yet been determined. However, important for any interpretation of these results is the fact that since MB is a penetrating dye it must have exerted its direct effects on either the muscle fiber membrane, the inner contractile system, or the mechanism that couples them, or on some combination of these. Work in progress is designed to localize these possible sites of action.

Effects of Acridine Orange and Zinc. The dye, in a concentration of 10^{-3} M in Ringer's solution, was studied in a procedure like that used for MB. Photodynamic effects somewhat similar to those involving MB were observed, but details of such observations will be given elsewhere. Of interest here are the following special effects that appeared, even in the total absence of light, during the initial period of soaking the muscle in the AO solution. (1) The peak tension produced in response to a single maximal shock progressively increased for the first 20 to 30 min. to a maximum which was on the average about 80% (in one case 150%) greater than the normal control. (2) These augmented responses were shown to be potentiated twitches by the demonstration that each was accompanied by a single action potential. (3) Following the period of increasing output, the potentiation gradually disappeared. (4) While the twitch was varying as just indicated, the tetanus output gradually decreased and the ability of the muscle to maintain its tetanus plateau progressively diminished. For reasons given in the introduction, experiments were now done with $ZnCl_2$ in 10^{-3} M concentration replacing the AO, and the results, insofar as present analysis indicates, were generally identical with those produced by the dye. We therefore conclude that the non-photodynamic effects of AO are simply due to its zinc content.

The most striking feature of the effects of Zn is its ability in very small concentration to greatly potentiate the twitch. No other inorganic ions yet studied approach this power of Zn (for effects of K, see Sandow and

Kahn (1952); and for anionic effects, see Kahn and Sandow (1955)). Furthermore, Zn, while exerting this intense twitch potentiation, causes a decrease in tetanus output, i.e., a decrease in maximal active state intensity, which is not characteristic of the aforementioned potentiating ions. Other evidence that cannot be given in detail here indicates that Zn potentiates the twitch by a direct action on the muscle fiber membrane which leads to a prolongation of the active state of the excited muscle. In explanation of the later appearing, suppressive effects on contractility, we hypothesize that these develop when Zn has penetrated into the fibers and acts directly on the contractile protein.

Future reports will deal in detail with the significance of our results for elucidating mechanisms of muscular response.

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