CARNOSINE AND TETRAMETHYLAMMONIUM ION AND ATP-INDUCED SHORTENING OF GLYCEROL-TREATED MUSCLE FIBERS

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Recently, it has been reported that tetramethylammonium chloride causes contraction of acetylated glycerol-treated muscle fibers while small metallic cations cause little or no contraction (Bowen and Martin, 1964). This led to speculation concerning the effect of large cations on the ATP-induced contraction of normal glycerinated muscle fibers.

Several large cations have been tested and will be discussed in forthcoming publications. The present report concerns the effect of tetramethylammonium ion (TMA) and carnosine on the ATP-induced contraction of glycerinated muscle fibers. Carnosine is a constituent of vertebrate muscle and exists in rabbit to the extent of about 0.6 percent (Severin, 1964).

METHODS

TMA and carnosine were applied to glycerinated muscle fibers as TMAATP or carnosine ATP which were made by neutralizing adenosine triphosphoric acid with them. Substitution for Na^+ and K^+ in the ATP molecule was done by converting Na_2ATP to adenosine triphosphoric acid with Dowex 50 (H cycle) and then neutralizing the acid with TMAOH or carnosine [1/]. Na_2ATP was also neutralized with carnosine which made the carnosine about 45 mM when ATP was 10 mM.

1/ The pK of carnosine equals 6.83 (Greenstein and Winitz, 1961) and the pH of solution equals 8 which means carnosine is zwitterionic with the imidazole present as free base.

Dowex 50-treated glycerinated fibers were prepared by adding the resin to the 50-50 glycerol-water mixture in which the fibers were being stored. They were treated for two weeks with occasional stirring before use. The pH of the mixtures was between 3 and 4.

The effect of these compounds and salts of ATP was tested by determining their effect on isotonic ATP-induced shortening of fine (150-200 μ wide) bundles of glycerol-treated rabbit psoas muscle fibers in about 0.4 ml of 6 or 10mM ATP solution with or without K^+, Mg^{++} or carnosine. The solution was spread out on a microscope slide lying on a metric scale. The initial and shortened lengths were measured on the scale by extending the fibers to straight but not stretched lengths at regular time intervals until shortening was completed. The amounts of shortening were taken as $\frac{\text{initial length} - \text{length at time } t}{\text{initial length}} \times 100$ and were plotted against the elapsed time as in the accompanying figures.

RESULTS

Application of Na_4ATP in which the quantity of other metallic ions had been reduced by resin treatment caused only slight shortening, but the inclusion of 1 mM $MgCl_2$ enhanced the rate of shortening to that shown (Fig. 1). Tetramethylammonium ATP (5 mM) was many fold more effective than Na_4ATP and the inclusion of 1 mM $MgCl_2$ enhanced the effect still more (Fig. 1). The application of carnosine Na_2ATP (carnosine about 45 mM compared to 30 mM in rabbit muscle (Severin, 1964)) increased the amount of shortening 50 fold over that accomplished with Na_4ATP and 10 fold over that with Mg during the initial 5 sec.

Adenosine triphosphoric acid which had been neutralized completely with carnosine ([carnosine] = 0.9 [ATP]) was much more effective than that neutralized with NaOH (cf. Figs. 1 and 2). The rates of shortening when 0.1 M carnosine was added to 6 mM Na_4ATP and when it was incorporated into ATP by neutralizing adenosine tri-

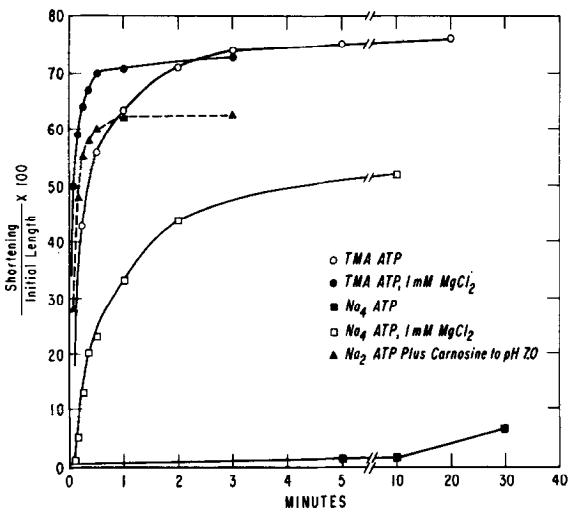

Fig. 1. Shortening (length lost) of fine bundles of glycerol-treated muscle fibers by adenosine triphosphoric acid neutralized with tetramethylammonium hydroxide or NaOH and by Na_2ATP neutralized with carnosine. [ATP] = 0.01 M; [carnosine^2] = about 0.009 M. All solutions pH 7.

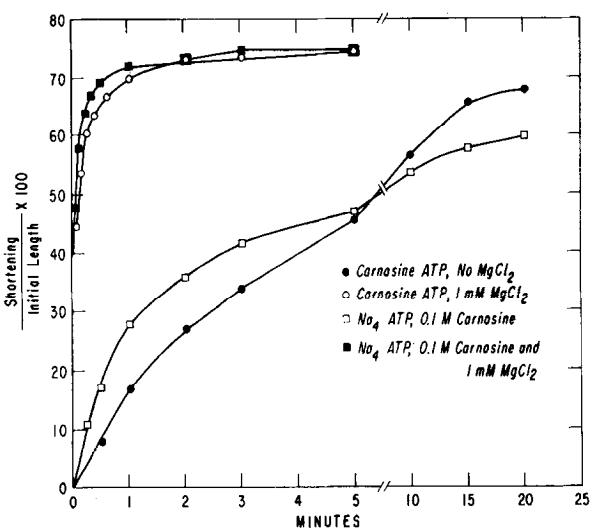

Fig. 2. Shortening (length lost) of fine bundles of glycerol-treated muscle fibers by adenosine triphosphoric acid neutralized with carnosine (final [carnosine] = 0.0054 M) and by NaOH. [ATP] = 6 mM. All solutions pH 7.

phosphoric acid with it were similar (two lower plots, Fig. 2). The slight difference could be due to increase in ionic strength contributed by Na^+. Carnosine ATP with 1 mM $MgCl_2$ was more effective in inducing contraction of the fibers than without Mg^{++}. The rate of shortening with Mg^{++} was virtually the same as that induced by tetramethylammonium ATP with Mg^{++} (Fig. 1).

In the experiments of Fig. 1, the solutions were between 0.10 and 0.11 ionic strength (μ). In the solutions of the experiments of Fig. 2, in which [ATP] was 6 mM, μ was < 0.05, except when 0.1 M carnosine was added. Then μ was increased by approximately 0.05. It is apparent therefore, that the effects of TMA and carnosine (Fig. 1) on ATP-induced shortening of the muscle fibers are independ of μ. The pronounced effect of carnosine ATP (Fig. 2) compared to the lack of effect of Na_4ATP (Fig. 1) was certainly not due to higher μ in the carnosine ATP solution. More extensive experiments in progress substantiate this conclusion and show without question that the carnosine effect is specific. Similar conclusions can be made about the effect of histidine and imidazole. These findings will be reported in the afore-mentioned subsequent publications.

These effects of carnosine have also been obtained in experiments in which both Dowex 50-treated glycerinated fibers and Na_4ATP were used. Addition of 5 mM ATP alone caused only slow shortening, but addition of 0.1 M carnosine accelerated the initial phases 10 to 20 fold (Fig. 3). Addition of 5 mM $MgCl_2$ and 0.1 M KCl to 5 mM Dowex-treated ATP accelerated the early phases of shortening over the rates without Mg^{++} and K^+. The addition of carnosine with Mg^{++} and K^+ produced 60 percent shortening in one minute compared to 12 percent without it.

These experiments indicate that tetramethylammonium ion and carnosine markedly augment the ATP-induced shortening of glycerol-treated muscle fibers. Attention is to be called to Severin's recent presenta-

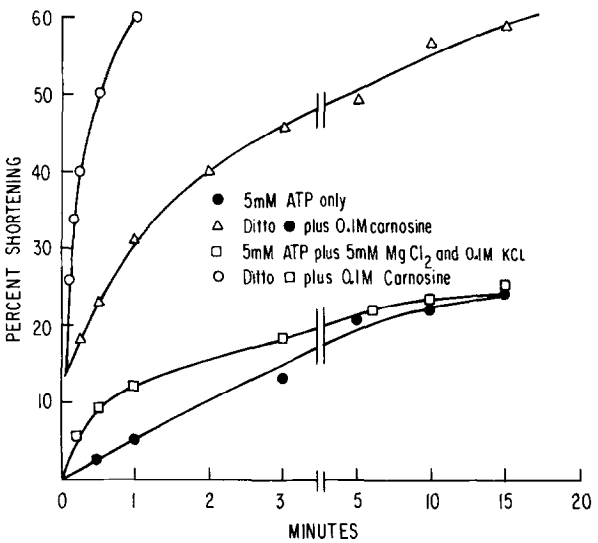

Fig. 3. Shortening of fine bundles of Dowex 50-treated glycerinated muscle fibers by the application of 5 mM Dowex 50-treated ATP with and without carnosine, KCl and $MgCl_2$. All reaction media were pH 8.

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tion (1964) in which he states that the number of SH-groups per gram of wet skeletal muscle is 10^{18} to 10^{19} while that of histidine dipeptides is within the range of 10^{18} to $3x10^{19}$. Severin (1964) has presented a great deal of data suggesting that carnosine and other imidazole derivatives in skeletal muscle may perform roles in anaerobic glycolysis, oxidative phosphorylation and neuro-muscular transmission. Also, its appearance in developing animals coincides with the appearance of the response of actomyosin to ATP. The present study indicates that carnosine directly augments the contraction of myofibrillar proteins and that this effect is specific and not related to its contribution of ionic strength or buffering action.

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