Relaxation in Glycerinated Cardiac Fibers* (29667)

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Recent studies by Parker and Berger(1) led to the conclusion that relaxation in cardiac muscle produced by cardiac relaxing factor granules and the nonphysiological relaxing factor ethylenediamine tetraacetic acid (EDTA) was unaffected by calcium ions. In these studies, the inhibition of a Mg-activated cardiac myofibrillar ATPase activity was used to indicate relaxation. Evidence will be presented here that EDTA and the more specific calcium chelating agent ethylene glycol bis (β-aminoethyl-ether)-N, N-tetracetic acid (EGTA)(2) produce a calcium reversible relaxation in glycerinated cardiac fibers under experimental conditions similar to those used by Parker and Berger. These results have been interpreted to suggest that inhibition of Mg-activated cardiac myofibrillar ATPase activity is not a measure of relaxing activity under the conditions used by these authors.

Materials and methods. Hearts from dogs under pentobarbital anesthesia were removed rapidly and placed in ice. Small strips were cut from tubercular muscle of the left ventricle, tied at resting length on wooden applicator sticks and placed in 50% glycerol (v/v) with 10 mM Tris, 10 mM Histidine, buffer (pH 7.0). The preparations were kept at 4° for 48 hours and the glycerol solution was changed every 24 hours prior to storage at —20°. The fibers were used 2-14 days after preparation. Small fiber bundles were dissected and isometric contractions measured as described previously(3). The control solution in which the fibers developed tension contained 50 mM KCl, 20 mM Tris, 2.5 mM oxalate, 5 mM ATP, and 5 mM magnesium (pH 7.0). All experiments were carried out at room temperature, 24°. The disodium salt of ATP, EDTA and EGTA was used and neutralized to pH 7.0 with sodium hydroxide. Water twice distilled from glass was used in all solutions.

Results. Fig. 1 is a reproduction of a typical polygraph recording of the effects of EDTA (EGTA gives similar results). Note that when ATP is added there is a rapid contraction, if EDTA (2 mM) is then added relaxation occurs (dotted line) which can be completely reversed by addition of equal molar concentrations of calcium (2 mM). Note that the addition of calcium without the presence of EDTA has no effect (solid

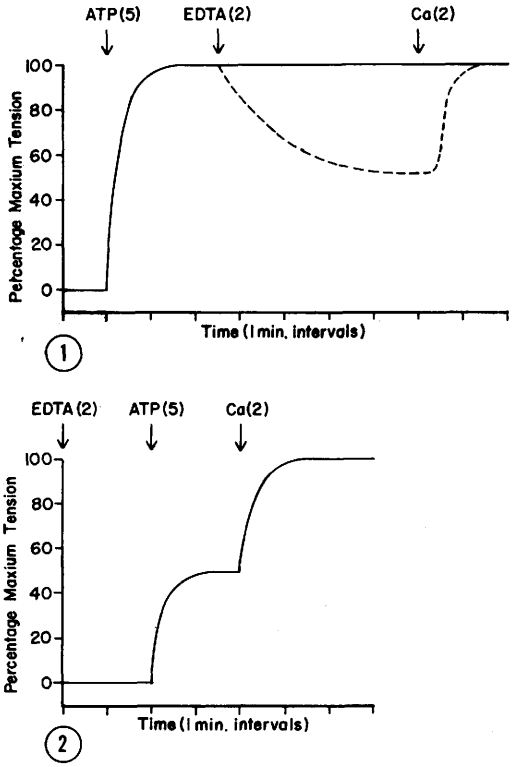

FIG. 1. Effects of EDTA and calcium on contraction induced by ATP and magnesium in glycerinated cardiac fibers. Concentrations (mM) are given in parentheses. Dotted line indicates effects of EDTA. For details, see *Results*.

FIG. 2. Effects of EDTA and calcium on contraction induced by ATP and magnesium in glycerinated cardiac fibers. EDTA was added prior to ATP. Concentrations (mM) are given in parentheses. For details, see *Results*.

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TABLE I. Effects of EDTA and EGTA on Inhibition of Contraction in Glycerinated Cardiac Fibers. The conditions are described under *Results*. % Inhibition is expressed as mean ± SEM. Number of experiments given in parentheses.

Conditions	% Inhibition	
EDTA	(9) 47 ± 3.7	p = .2-.3
EGTA	(5) 35 ± 6.4	

line). In Fig. 2, EDTA has been added prior to addition of ATP. Note that maximum contraction occurs only upon addition of calcium. Table I shows the effects of EDTA (2 mM) and EGTA (2 mM) in a series of fibers on the percentage inhibition as compared to the maximum tension developed when 2 mM of calcium is subsequently added. In these experiments the relaxing agent is added 5 minutes before addition of ATP; calcium (2 mM) is added after the tension has reached a plateau as shown in Fig. 2. A mean of 47% and 35% inhibition occurs in 2 mM EDTA and EGTA respectively. The difference is not statistically significant (p=0.2-0.3).

Discussion. In a recent comparison between the so-called relaxing factor systems from heart and skeletal muscle, Parker and Berger(1) concluded that the systems were interchangeable since they both inhibited the Mg-activated ATPase activity of either cardiac or skeletal myofibrils. However, inhibition of cardiac myofibrillar ATPase activity by either the relaxing factor granules or a nongranular relaxing factor EDTA was not reversed by calcium. It was concluded therefore that, since calcium has no effect on inhibition of cardiac myofibrillar ATPase activity by the various relaxing factors, the relaxing factors do not act by binding calcium which is essential for ATPase activity. In the present study, however, using similar solutions and concentrations of nonphysiological relaxing agents as used by Parker and Berger, it can be readily demonstrated that EDTA and the more specific calcium chelating agent EGTA do produce a significant relaxation in glycerinated cardiac fibers which is calcium reversible. Thus, if the relaxing agents were added after the maximum contraction induced by ATP, relaxation occurred which could be completely reversed by calcium (Fig. 1). If added before ATP (Fig. 2), EDTA and EGTA produced a 47% and 35% inhibition of contraction respectively, as compared to the maximum contraction obtained when 2 mM calcium was subsequently added (Table I). These results indicate that under the conditions of these experiments the nonphysiological relaxing factors (EDTA and EGTA) produce a calcium reversible relaxation in glycerol extracted cardiac fibers. This suggests that inhibition of Mg-activated cardiac myofibrillar ATPase activity under the conditions used by Parker and Berger does not represent a model for relaxation. It is possible, however, that the cardiac myofibrils unlike skeletal myofibrils may contain a contaminating Mg-activated ATPase which is not calcium dependent and not associated with contraction. Thus the conclusion that cardiac relaxing factor granules or the nongranular relaxing factor EDTA do not act by binding calcium is not justified at present. Furthermore, since there is no evidence that EDTA(4) and EGTA† are bound to the contractile elements in any significant amounts, it is possible that they produce relaxation by inactivating calcium contaminating the fibers and solutions. The binding of Mg does not appear to play any significant role in this process since both EDTA and EGTA act similarly. Under the conditions of our experiments the binding of Ca by these agents is similar but the binding of Mg by EDTA is negligible(5).

Summary. The effects of the nonphysiological relaxing agents EDTA and EGTA on contraction induced by ATP and magnesium in glycerol extracted dog cardiac fibers were investigated. Both EDTA (2 mM) and EGTA (2 mM) produced a 47% and 35% inhibition of contraction respectively as compared to the maximum tension developed when calcium (2 mM) was subsequently added. It was concluded that since these chelating agents produce a calcium reversible relaxation in glycerinated cardiac fibers,

† Personal communication from Dr. F. N. Briggs of the University of Pittsburgh.

measurements of inhibition of Mg-activated ATPase in cardiac myofibrils under similar conditions is not a model for relaxation as suggested in the literature. Cardiac myofibrils may therefore contain a contaminating Mg-activated ATPase which is not Ca dependent and not associated with contraction.

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Regulation of Plasma Fluoride in Rats.* (29668)

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The constancy of fluoride content of plasma of humans using communal waters with a wide range of fluoride concentration has been reported(1). The mean plasma fluoride concentration of hospitalized patients was not different from that of normal individuals. The only circumstance in which there was a suggestion of higher than normal plasma fluoride values was in 3 cases of demineralizing bone disease. Brzezinski, Gedalia, Danon, and Sulman(2) reported no difference between the fluoride contents of the blood of pregnant and non-pregnant rats. Their data also suggest that large variations in aqueous fluoride intake did not appreciably affect the blood fluoride concentration.

The present investigation was designed to examine the effects of dietary and physiological variables which might influence the plasma fluoride concentration. The factors of fluoride intake, skeletal fluoride content, starvation and parathormone administration have been studied to determine the ability of the homeostatic mechanisms controlling the plasma fluoride concentration to cope with these stresses.

Experimental. Male Sprague-Dawley rats were used. All animals were fed *ad libitum* Purina Laboratory Chow which contained 60-85 ppm of fluoride and received varying intakes of fluoride *via* the drinking water. The animals were bled by heart puncture using specially washed equipment to eliminate fluoride contamination(1), and heparin was used as an anticoagulant. The bones were cleaned of soft tissues, freed of marrow, dried, and fat-extracted with an equal-part mixture of absolute alcohol and ethyl ether for 48 hours before being ashed at 500°C. The blood plasma samples and the ash of the humeri were analyzed for fluoride by the microdistillation method of Singer and Armstrong(3). Analyses for plasma calcium were carried out by the method of Buchner and Shively(4).

Study I. Relation between skeletal and plasma fluoride contents. In this study 93 of a total of 106 rats, approximately 60 days of age, were divided into 4 sub-groups (Table I, Part 1). They were fed laboratory chow containing 60-70 ppm of fluoride and were given distilled water containing either 0, 10, 30, or 50 ppm of fluoride. This regimen was continued for 60 days and the animals were then given distilled water for 30 days before being sacrificed. The other 13 animals of the study (Table I, Part 2) were given laboratory chow with a slightly higher fluoride content (85 ppm) than that received by the animals described above and were maintained on either distilled water or distilled water plus 50 ppm of fluoride until time of sacrifice. These animals were killed after 60 days on the dietary regimen.

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