Magnesium and the regulatory role of "calcium-sensitizing factor" in muscle.

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Following on certain observations on the action of metin (1) it appeared likely that magnesium would be necessary for calcium-sensitization of actomyosin by "native tropomyosin" (2,3) which contains tropomyosin and troponin (4). The present findings show that this is indeed the case.

"Native tropomyosin" (calcium-sensitizing factor) was prepared (3) dialyzed and its effect was studied on superprecipitation and ATPase

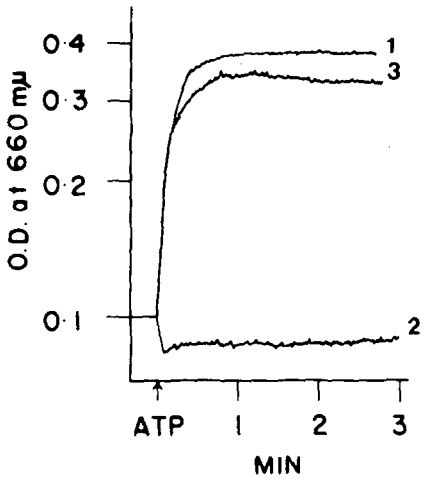

Figure 1. Effect of $MgCl_2$ on the inhibitory action of Ca-sensitizing factor on superprecipitation of actomyosin. Changes in optical density (OD) were measured with a Cary recording spectrophotometer at 660 mμ. The graphs were retraced to read from left to right. The cuvette contained 2.2 ml of the following: synthetic actomyosin about 1.0 mg (actin/myosin = 1/4 by wt), tris-maleate buffer (1.6×10^{-2}M, pH 6.8), KCl (5×10^{-2}M) EGTA (2.5×10^{-5}M), and ATP (5×10^{-4}M neutralized with NaOH) was added at the point marked with arrow (see 1). In 2, the reaction mixture was the same as 1 but also contained Ca-sensitizing factor (330 μg) and $MgCl_2$ (10^{-3}M); addition of ATP resulted in "clearing." In 3 the ingredients were the same as for 2 except for the absence of $MgCl_2$; superprecipitation is not inhibited.

activity of synthetic actomyosin. No attempt was made to remove any traces of magnesium which are present in the ingredients of the reaction mixtures.

Figure 1 shows that superprecipitation is not inhibited by ethylene dioxybis(ethylene amino) tetra-acetic acid (EGTA) in the presence of calcium-sensitizing factor if $MgCl_2$ is not added to the medium. Likewise, EGTA and calcium-sensitizing factor do not inhibit ATPase activity significantly if $MgCl_2$ is not added; in the presence of $MgCl_2$ there is inhibition, the degree of which is dependent on the concentration of $MgCl_2$ as well as on the concentration of calcium-sensitizing factor (Fig.2a). The specific ATPase activities, depicted as 100% in Fig.2a are, as expected, not comparable at the varying concentrations of $MgCl_2$; without adding $MgCl_2$ the initial specific activity (i.e., prior to the addition of calcium-sensitizing factor) is comparatively low. Hence, the possibility arose that EGTA and calcium-sensitizing factor could not inhibit a relatively inactive enzyme irrespective of the lack of $MgCl_2$. To rule out this possibility, the initial ATPase activity in the presence of $MgCl_2$ was lowered by increasing the KCl concentration in the medium. Thus, the activity was lowered to a level comparable with that obtained without $MgCl_2$ and once again EGTA and calcium-sensitizing factor inhibited ATPase activity (Fig.2b).

Thus, it is demonstrated that calcium sensitization of actomyosin is dependent on the concentration of magnesium. It should be noted that using an actin preparation from a different "actin-powder" resulted, subsequently, in a greater degree of ATPase inhibition in each experiment including the one without added $MgCl_2$. Dr. D. J. Hartshorne kindly tested their preparation (5) of calcium-sensitizing factor and showed a similar dependence on $MgCl_2$ (personal communication). This direct evidence on the relationship of magnesium to calcium sensitization of actomyosin is of relevance in the interpretation (6) of previous findings on the inhibitory effect of magnesium on ATPase and superprecipitation of myosin B (7) and myofibrils (8), preparations which are now known to contain tropomyosin and troponin. Further

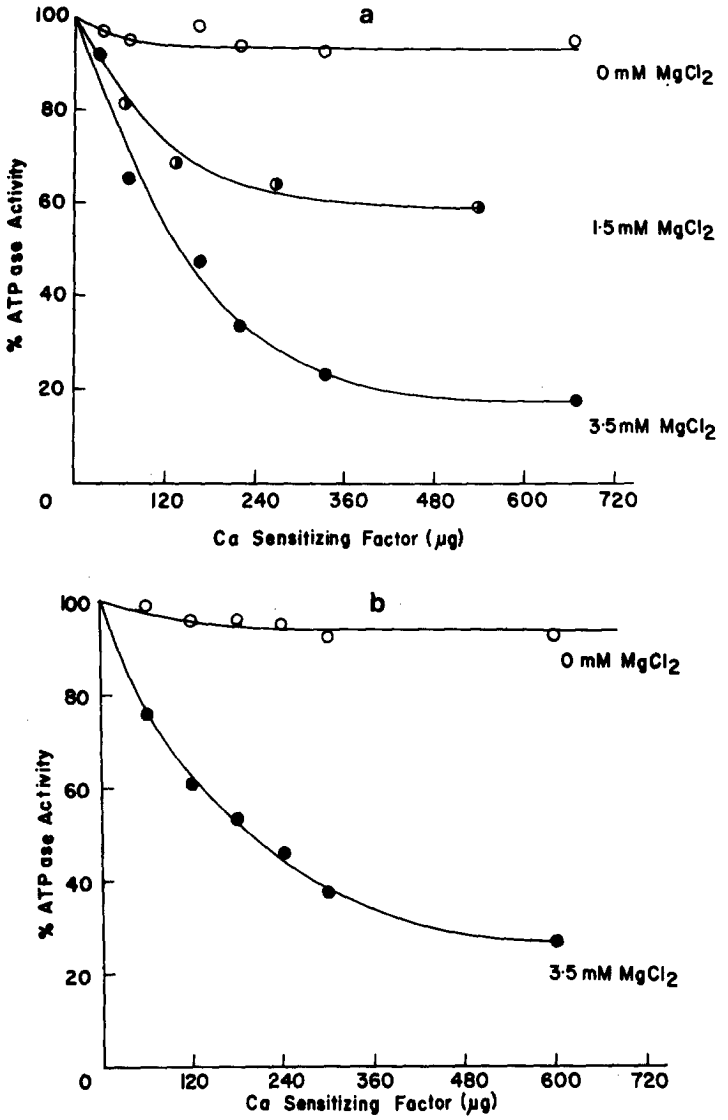

Figure 2. Effect of $MgCl_2$ on the inhibitory action of varying conc of Ca-sensitizing factor on ATPase activity of actomyosin. The reaction mixture (2.3 ml) contained 0.9 mg of synthetic actomyosin (actin/myosin = 1/4 by wt.), EGTA (8.7×10^{-5} M), tris-HCl buffer (1.5×10^{-2} M, pH 8.0), varying amounts of Ca-sensitizing factor as indicated and 3.5×10^{-3} M ATP (neutralized with NaOH). In (a) the KCl conc was 16×10^{-3} M and the specific activities (without Ca-sensitizing factor) depicted as 100% were: 0.6 µmoles P/mg actomyosin/5 min in 0 $MgCl_2$ (○); 3.2 µmoles P/mg actomyosin/5 min in 1.5×10^{-3} M $MgCl_2$ (◑); and 3.4 µmoles P/mg actomyosin/5min in 3.5×10^{-3} M $MgCl_2$ (●).

In (b) the specific activity (without Ca-sensitizing factor) depicted as 100% was 0.9 µmoles P/mg actomyosin/5 min in 0 $MgCl_2$ & 16×10^{-3} M KCl (○) and 1.0 µmoles P/mg actomyosin/5 min in 3.5×10^{-3} $MgCl_2$ & 64×10^{-3} M KCl (●); these specific activities, in contrast to (a), are comparable. The reaction mixtures were incubated in a water bath at 25°C.

experiments on purified actomyosin and these regulatory proteins may lead to a better understanding of the specific sites and role of magnesium as well as of calcium (which binds to troponin (9,10)) in the contraction and relaxation processes.

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