Essential Relaxing Factor in Muscle other than Myokinase and Creatine Phosphokinase

Extracts of rabbit skeletal muscle contain factors which play a part in the relaxation of muscle fibre models, such as glycerinated fibres[1–3]. Two of them isolated have been identified with known enzymes: myokinase by Bendall[4] and creatine phosphokinase by Lorand[5]. But these two enzymes, coupled with their co-factors, do not settle all the problems of muscular relaxation because, in the light of our experiments, they do not have any relaxing effect on glycerinated fibres which have been preserved for a long time and exhaustively washed (Fig. 2*a*).

We have shown[3] that the adenosine triphosphate-dependent relaxing factor, in fact, consists of two different components; one occurs in the fraction precipitated by ammonium sulphate at concentrations of 10–20 gm. of sulphate per 100 ml. of extracted solution from muscle, tentatively named 'fraction *A*', and the other in the fraction precipitated by concentrations of 30–40 gm. of sulphate per 100 ml. of extracted solution from muscle, 'fraction *B*'. Each has a moderate relaxing effect on relatively fresh fibres. If the fibres to be tested, however, have been preserved in 50 per cent aqueous glycerol for one month or more at − 10° C. and washed in 0·1 *M* potassium chloride solution for several hours, each fraction by itself no longer has a relaxing effect (Fig. 1, *a* and *b*). The relaxing activity is not recovered until these two fractions are used together (Fig. 1*c*).

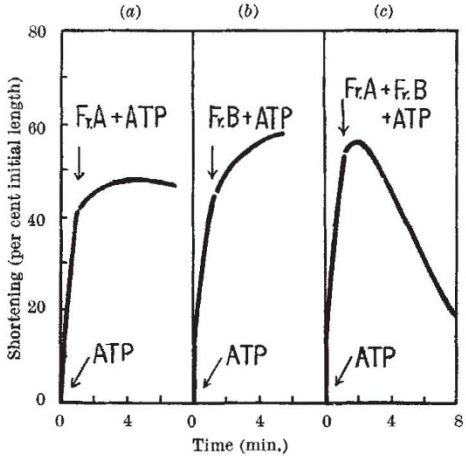

Fig. 1. Effect of fraction *A* and *B* on a muscle fibre shortened in adenosine triphosphate.

ATP = 5 m*M* adenosine triphosphate; Fr. *A* + ATP = 2·2 mgm./ml. of fraction *A* + 5 m*M* adenosine triphosphate; Fr. *B* + ATP = 17 mgm./ml. of fraction *B* + 5 m*M* adenosine triphosphate; Fr. *A* + Fr. *B* + ATP = 2·2 mgm./ml. of fraction *A* + 15 mgm./ml. of fraction *B* + 5 m*M* adenosine triphosphate. All the experiments were performed in 0·15 *M* potassium chloride containing 0·01 *M* magnesium chloride. For fractions *A* and *B* see text

Fraction *A* is highly opalescent, suggesting that it contains a considerable amount of lipid. It has strong adenosine triphosphatase activity, which corresponds apparently to Kielley–Meyerhof's adenosine triphosphatase[7]. No method has so far been found for separating the active relaxing principle from the enzyme. These two substances are quite similar in properties in certain respects: both can be centrifuged at 18,000 *g* for 1 hr. without losing their activity; both are activated by magnesium ions, the optimum of which is about 0·004 *M*, and inactivated specifically by a small amount of calcium ions. Their identity, however, remains to be proved and requires further study.

Fraction *B* contains myokinase and creatine phosphokinase. It is probable that myokinase plays an important part in the adenosine triphosphate-dependent relaxing action of fraction *B*. Indeed, crude myokinase, if combined with fraction *A*, has a lengthening effect on moderately washed fibres (Fig. 2*b*). Nevertheless, the whole activity of fraction *B* cannot be ascribed to myokinase, for this fraction is far more labile to acid than myokinase, and in the case of old and washed fibres, myokinase is ineffective, even when combined with fraction *A* (Fig. 2*c*). Some other factor or factors in fraction *B* evidently participate in relaxation (Fig. 2*d*).

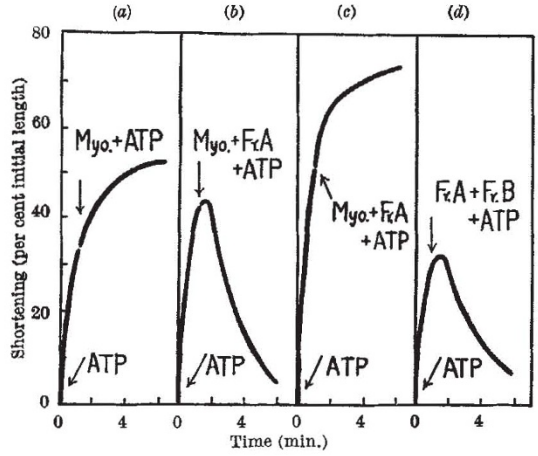

Fig. 2. Effect of crude myokinase and fraction *B* combined or uncombined with fraction *A* on a muscle fibre shortened in adenosine triphosphate.

Myo. + ATP = 0·4 mgm./ml. of crude myokinase (acidified and fractionated by ammonium sulphate) + 5 m*M* adenosine triphosphate; Myo. + Fr. *A* + ATP = 0·16 mgm./ml. of crude myokinase + 5 mgm./ml. of fraction *A* + 5 m*M* adenosine triphosphate; Fr. *A* + Fr. *B* + ATP = 5 mgm./ml. of fraction *A* + 8 mgm./ml. of fraction *B* + 5 m*M* adenosine triphosphate. In (*a*) and (*b*) relatively unwashed muscle fibres were used; (*c*) and (*d*) fibres preserved and washed. Other notes as in Fig. 1

Unless it is aided by fraction *A*, the creatine phosphokinase–phosphocreatine–adenosine triphosphate system[2,5] also has no effect on old and washed fibres.

As described above, fraction *A* is essential for the relaxation of muscle fibre models. Experiments by other workers[1,2,4,5] have probably been conducted on fibre preparations which still contain some of the active principle of this fraction.

Details of this work will be published elsewhere.

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