ON THE EFFECT OF INHIBITING THE ACTIN-MYOSIN INTERACTION ON THE VISCOUS TONE OF A LAMELLIBRANCH CATCH MUSCLE

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The 'catch' or the viscous tone, a reversible rigor of tonic lamellibranch muscles such as the anterior retractor of the byssus in Mytilus edulis (ABRM), may be due to a stiffness in its actomyosin contractile system resulting from bonds between actin and myosin elements (Lowy and Millman, 1959, Hanson and Lowy, 1961). Alternatively a mechanical system lying in parallel to the actomyosin contractile system may serve to hold 'catch' tension (Rüegg, 1958, 1961: Johnson et al. 1959). The existence of such an independent holding apparatus seems probable since glycerinated ABRM fibres tension can still be supported after treatment of the fibres with salyrgan or with EDTA or other interaction inhibitors of the type described by Barany and Jaisle (1960); these agents are known to inhibit the actin-myosin interactions in glycerinated rabbit psoas muscle and in gels of rabbit actomyosin. The present paper describes evidence showing that in the surviving ABRM as well as in its glycerinated fibres the actin-myosin interaction can be inhibited by the same inhibitor. It thus becomes possible to find out whether or not the 'catch' tension can be supported in the intact i.e. living ABRM under conditions in which the actomyosin system is relaxed.

Encouraged by the work of Barany et al. (1960) and also by unpublished results of Portzehl, thiourea has been tested as a possible interaction inhibitor in vitro: In concentrations of 0.4-0.5 M it reduces the Mg-activated actomyosin ATP-ase of purified ABRM actomyosin or of blendor-treated washed ABRM fibres by about 80 % while the Ca- activated ATP-ase as well as the Mg- activated ATP-ase of muscle particles contaminating the actomyosin ATP-ase is not inhibited and may even be enhanced in the presence of 0.5 M thiourea. The assay conditions were 0.05 M KCl, 0.05 M histidine buffer pH 7, 0.005 M Ca or Mg and 0.005 M ATP, 25°C. The active tension production in glycerinated ABRM fibres is innibited totally, and fairly reversibly, by 0.4-0.5 M thiourea (0.1 M KCl, 0.02 M histidine pH 7, 0.005 M Mg, ATP). As in the case of other interaction inhibitors (cf. Barany and Jaisle, 1960) the Mg- activated actomyosin ATP-ase as well as the tension production in glycerinated fibres is, however, very little inhibited by 0.5 M thiourea if the ATP concentration is less than 0.5 mM. If 0.5 M thiourea is added after an ATP induced contraction the glycerinated ABRM fibre bundle relaxes only by about 50 % in the first 10 minutes although the ability to redevelop tension after a quick release is totally inhibited. The contraction remainder of the extracted muscle fibre would not appear to be due to a failure of its actomyosin system to relax completely in thiourea since it is about the same as the contraction remainder after addition of EDTA or salyrgan; and salyrgan has been shown to produce a complete and reversible relaxation of an ABRM actomyosin thread after an ATP induced contraction. Experiments in which the contraction remainder has been compared with certain

properties of artificial tropomyosin A threads have shown that the tension surviving the inactivation of the actomyosin system by salyrgan or EDTA is supported by the tropomyosin-paramyosin system of these muscles (cf. Rüegg, 1961). It thus follows that thiourea has very little effect on this holding system of glycerinated fibres in concentrations in which it inhibits the actomyosin contractile system completely. Urea on the other hand affects holding apparatus and contractile mechanism at the same time.

If a surviving ABRM is incubated in seawater containing 0.6 M thiourea it becomes, within about 15-20 minutes, almost completely penetrated by the interaction inhibitor. The half-time of influx and outflux is about 4 minutes at room temperature and 8 minutes at 10°C. After penetration into the ABRM, thiourea in the concentration mentioned inhibits fairly reversibly all contractile responses regardless of whether the muscle is stimulated by direct current, acetylcholine (0.5 mM), isotonic KCl or 0.3 % caffeine. There is evidence that thiourea acts directly on the actomyosin contractile system as it must do in the case of glycerol extracted fibres. Unpublished experiments (with Drs. Betty Twarog and R. Straub) using the sucrose gap technique have shown that thiourea has a differential action on the muscle membrane and on the contractile apparatus: Acetylcholine and KCl still depolarise the membrane in the presence of thiourea though they no longer evoke contraction. Since the caffeine contracture is also inhibited by thiourea it seems probable that the point of action of thiourea is either the contractile mechanism itself or one of the last links in the excitation-contraction coupling. Moreover the

dependence of the inhibition on the concentration of thiourea is nearly the same in the surviving ABRM and in its glycerinated fibres. The action of thiourea cannot be due to unspecific effects (e.g. osmotic effects) common to both thiourea and urea since the latter inhibits neither the surviving ABRM nor its extracted fibres in a concentration of 0.6 M. Thiourea, therefore, appears to have, after its penetration into the living muscle cell, a similar action on the actomyosin contractile apparatus as it has in extracted fibres where it acts by inhibiting the actin-myosin interaction.

After 30 minutes incubation in thiourea seawater (0.6 M thiourea, 10°-12°C, pCO_2 100 mm Hg, pH 6.8 phosphate-bicarbonate buffer) the catch phenomena can still be demonstrated although active contraction is no longer possible. At 50 % body length of the muscle the 'resistance to stretching' (measured 4 minutes after quick stretch, defined according to Weber and Portzehl, 1952) is then about 2000-4000 g x cm^{-2} x L x ΔL^{-1}. The passive tension obtained on stretching the ABRM eg. 1000 g/cm^2 shows the characteristics of the 'catch'. In particular, it disappears after addition of 5×10^{-5}M serotonin and like the contraction remainder of extracted fibres and also like the 'catch' tension, it disappears under conditions which reduce the hydrogen ion concentration of the medium surrounding the structural proteins. In the case of the living muscle a reduction of internal hydrogen ion concentration can be produced by reducing the partial CO_2 pressure from e.g. 100 mm Hg to 0. These results show that under the reported conditions thiourea has a differential action on the ability to produce tension actively and on the ability to maintain 'catch' tension or viscous tone. To-

gether with the above mentioned biochemical and physiological findings our results strongly suggest that a functional actomyosin system is not needed to maintain serotonin sensitive passive tension and to resist stretch. It would appear then that the 'catch' is not (or at least not entirely) dependent on linkages responsible for the interaction of actin and myosin elements in the ABRM but on a system lying functionally in parallel to the actomyosin system. This system may well be identical with the holding apparatus previously studied in glycerol extracted fibres and found to be associated with tropomyosin A (paramyosin) which is, according to Bailey (1956), so abundant in these muscles.

A much more detailed report dealing also with other aspects of thiourea action will be published jointly by Drs.B.M.Twarog, R. Straub and the author. I am thankful to Prof. H. H. Weber for his constructive criticism throughout this work.

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