The influence of P-light chain phosphorylation by myosin light chain kinase on the calcium sensitivity of chemically skinned heart fibres

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Phosphorylation of the P-light chain of myosin might be involved in the regulation of cardiac contractility. Thus an enhanced phosphorylation level of the P-light chain catalyzed by Ca^{2+}-calmodulin-dependent myosin light chain kinase (MLCK) increased significantly the Ca^{2+} sensitivity of chemically skinned ventricular fibre bundles of the pig. This effect was reversible. Whereas force development at submaximal Ca^{2+} concentration (pCa 5.5) increased by ~ 50% in the presence of MLCK, maximum tension achieved at maximum Ca^{2+}-concentration (pCa 4.3) was not affected.

Myosin light chain kinase *P-light chain* *Phosphorylation* *Chemically skinned heart fibre*

1. INTRODUCTION

The P-light chain of cardiac, skeletal and smooth muscle myosin can be phosphorylated by Ca^{2+}-calmodulin activated myosin light chain kinase (MLCK) and dephosphorylated by a specific phosphatase [1]. Whereas in smooth muscle phosphorylation of the P-light chain is involved in the activation of contraction [2], its role in skeletal and cardiac muscle is still a matter of controversy.

In both skeletal and cardiac muscle myofibrils, the ATPase activity and the rate of cross-bridge cycling decreased after phosphorylation or thiophosphorylation when the myofibrils were fixed with glutaraldehyde [3,4].

Thiophosphorylation of chemically skinned cardiac fibres decreased the ATPase activity, tension cost, cross-bridge cycling rate [5,6] and V_{max} [5], while force development was not inhibited [5].

During the past few years, the influence of various factors on the calcium sensitivity of chemically skinned heart fibres has been the subject of many studies. Cyclic nucleotides, e.g. cAMP and cGMP, and increased H^+ concentration desensitized while some novel cardiotonic drugs sensitized skinned heart fibres for Ca^{2+} [7]. Recently, an increased isometric force and phosphorylation level of the P-light chain after MLCK treatment of chemically skinned skeletal muscle fibres at submaximal Ca^{2+} concentration has been reported [8]. Here, we focus on the effect of MLCK on the calcium sensitivity of chemically skinned heart fibres.

2. MATERIALS AND METHODS

Pig right ventricular fibre bundles were chemically skinned as described [9] except that 1% Triton X-100 was used as a detergent. The relaxation and contraction solutions both contained an ATP-regeneration system. The relaxing solution was composed of 10 mM ATP, 12.5 mM $MgCl_2$, 5 mM EGTA, 20 mM imidazole, 5 mM NaN_3, 10 mM phosphocreatine and 380 U/ml creatine phosphokinase (Boehringer), pH 6.7 (22°C). The contraction solution had the same composition as the relaxation solution, except that EGTA was substituted by 5 mM Ca-EGTA. In addition, all solutions contained 4.2 μM calmodulin dissolved

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in 10 mM Tris, 0.2 mM DTE, pH 7.0 (4°C) and 80 μl/ml MLCK-buffer (see below). Instead of MLCK-buffer, the test solution contained 80 μl of a 0.15 mg/ml MLCK preparation dissolved in 5 mM Mes, 0.2 mM benzamidine, 0.2 mM EDTA, 30 mM KCl, 0.5 mM DTT, 50% glycerol, pH 6.5 (4°C), resulting in a final concentration of 12.4 μg MLCK/ml test solution. Myosin light chain kinase was from bovine heart and prepared as described [10].

For mechanical measurements, the fibres were mounted isometrically and connected to a force transducer (AME AE 801). Force was recorded at pCa 5.5 in both the presence and absence of MLCK, respectively, and at pCa 4.3.

The P-light chain phosphorylation of skinned fibres incubated at pCa 5.5 in both the presence and absence of MLCK, respectively, was determined by two-dimensional gel electrophoresis as described [11].

The amount of phosphorylated and unphosphorylated light chain 2 was determined by scanning the Coomassie-stained spots. The fibres selected for mechanical experiments and P-light chain phosphorylation determination had been stored for 7 days at −20°C in 20 mM imidazole, 10 mM NaN_3, 5 mM ATP, 5 mM $MgCl_2$, 4 mM EGTA, 2 mM DTE, 50% glycerol, pH 7.0.

3. RESULTS

Fig.1 shows that the treatment of chemically skinned heart fibres with MLCK increased the force induced at submaximal Ca^{2+} concentration (pCa 5.5) but had little effect on maximal contraction at pCa 4.3. As demonstrated in the same figure, this MLCK-effect was reversible. After 2 contraction-relaxation cycles in both the absence and presence of MLCK, respectively, the fibre was incubated for 30 min in relaxation solution to wash out the enzyme. The subsequent contraction-relaxation cycle showed only a small decrease in the MLCK-effect, while in the second post-incubation cycle the MLCK-effect had disappeared completely: the isometric tension at pCa 5.5 returned to the control level while maximal force achieved at pCa 4.3 remained unchanged.

On average, submaximal Ca^{2+} concentration without MLCK induced 17.5 ± 5.3% of the isometric tension achieved at maximum activation (table 1). MLCK treatment of the fibres at pCa 5.5 increased the mean isometric force to 23.3 ± 4.7% of the tension achieved at maximum activation (table 1). This increase in force was statistically significant at the $p < 0.005$ level (Student's t-test for paired values). The SE values were high because the Ca^{2+} sensitivity of the individual fibre preparations differed from each other widely in spite of the identical skinning procedure.

Comparing directly the force generation of the fibres at pCa 5.5 in both the presence and absence of MLCK, respectively, taking the isometric force at pCa 5.5 without MLCK as 100%, the MLCK-induced increase of isometric force at pCa 5.5 was 47.5 ± 10% (mean ± SE, number of fibres in parentheses). The maximum isometric force at pCa 4.3 was nearly constant before and after MLCK treatment, only a slight increase of pCa

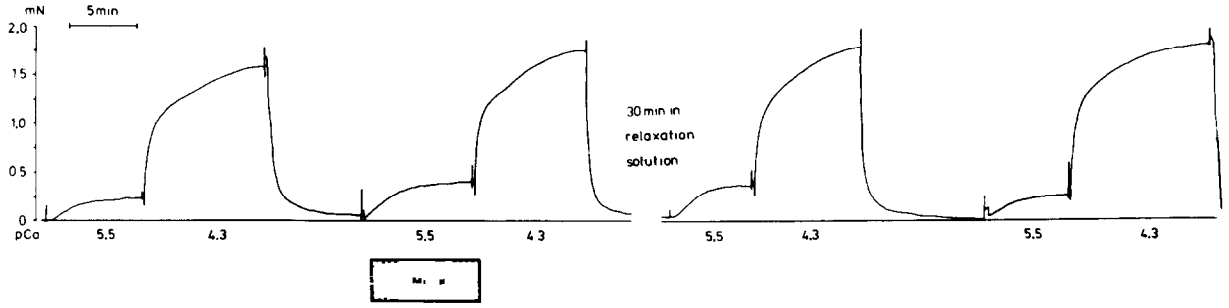

Fig.1. Effect of MLCK on the isometric tension of skinned heart fibres and the reversibility of this effect. The fibre shown in the figure was contracted at submaximal (pCa 5.5) and at maximal (pCa 4.3) Ca^{2+} concentration in the absence and in the presence of myosin light chain kinase (+ MLCK). After these 2 contraction-relaxation cycles the fibre was incubated for 30 min in relaxation solution to wash out the enzyme. After the incubation period, two additional contraction-relaxation cycles in the absence of MLCK were carried out.

Table 1

	Isometric tension	LC-2-phosphorylation ('P-LC-2'/LC-2)
− MLCK	17.5 ± 5.3% (6)	0.46 ± 0.06 (4)
+ MLCK	23.3 ± 4.7% (6)	0.81 ± 0.04 (4)

Influence of myosin light chain kinase (MLCK) on the isometric tension and on the LC-2 phosphorylation at submaximal Ca^{2+} concentration (pCa 5.5). Isometric tension is expressed as % of maximum tension achieved at maximum activation (pCa 4.3). LC-2 phosphorylation is expressed as the relation of phosphorylated P-LC-2 to unphosphorylated light chain 2 (LC-2). Values are means ± SE with the number of fibres in parentheses

4.3-induced isometric force after MLCK treatment of 4.4 ± 1.6% [12] could be observed (mean ± SE, number of fibres in parentheses).

A different set of fibres was treated in the same manner as the fibres for mechanical measurement at pCa 5.5 in both the absence and presence of MLCK, respectively, and fixed after 10 min with 15% trichloroacetic acid/2% PPa to determine P-light chain phosphorylation.

Three spots representing the P-light chain were visible after two-dimensional gel electrophoresis (fig.2). These spots have already been identified and designated LC-2, LC-2*, LC-2-P, and LC-2*-P [12,13]. As observed [13], LC-2* and LC-2-P migrate in the secod dimension as one component. This spot has been referred to as 'P-LC-2' in this article (fig.2). MLCK treatment of the fibres increased the relation P-LC-2 to LC-2 from 0.46 in the untreated to 0.81 in the MLCK-treated fibres at pCa 5.5 (table 1, fig.2). This corresponds to an increase in the phosphorylation level of LC-2 of 76%.

4. DISCUSSION

Treatment of chemically skinned heart fibres with MLCK increased the Ca^{2+} sensitivity and enhanced the P-light chain phosphorylation of myosin. The reversed reaction, namely Ca^{2+} desensitization, was achieved using smooth muscle myosin phosphatase which decreased the P-light chain phosphorylation (manuscript in preparation). Similar results have been reported recently

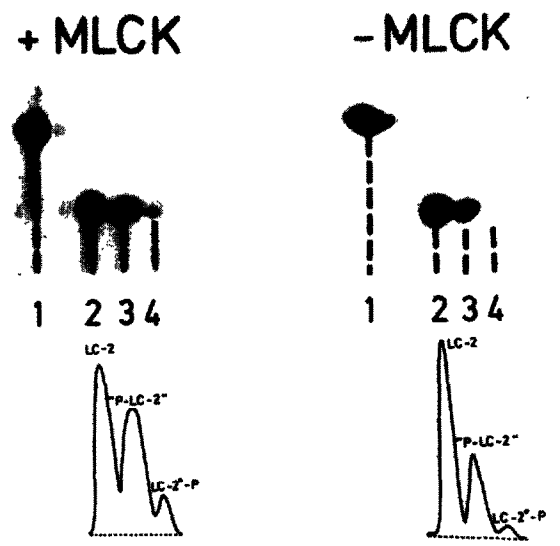

Fig.2. Two-dimensional gel electrophoresis of the light chains of pig ventricular skinned fibres. The procedure was carried out as described [3]. Top: the Coomassie-stained myosin light chain spots in the second dimension. Bottom: densitometric scan of the different forms of the P-light chain. 1 = LC-1, 2 = LC-2, 3 = P-LC-2, 4 = LC-2*-P.

for skeletal muscle fibres [8].

Phosphorylation of the P-light chain with ATP and ATPγ S (thiophosphorylation), respectively, provoked different reactions: thiophosphorylation of glutaraldehyde fixed myofibrils of cardiac [6] and skeletal muscle [3] and of skinned cardiac fibres [6] decreased ATPase activity and the rate of cross-bridge cycling while phosphorylation with ATP had no influence on these parameters [8].

The effect of P-light chain phosphorylation/dephosphorylation on the Ca^{2+} sensitivity of skinned heart fibres suggests a regulatory mechanism of cardiac contractility in addition to other regulatory systems which have already been described, e.g. troponin-I phosphorylation (review [7]) and myosin isoenzyme alteration [14,15]. As in the case of troponin-I phosphorylation catecholamine stimulation of the heart may be involved in P-light chain phosphorylation, because the catecholamine induced increase in intracellular Ca^{2+} and the subsequent binding of Ca^{2+} to calmodulin are prerequisites of MLCK activation [1,10].

Indeed, short-term perfusion with positive inotropic agents increased the P-light chain phosphorylation [16,17,18]. Enhanced P-light chain phosphorylation was positively correlated with increased cardiac active tension [17]. In other studies however, no influence of inotropic agents on cardiac myosin P-light chain phosphorylation could be demonstrated [19,20].

Furthermore, the enhancing effect of P-light chain phosphorylation on the Ca^{2+} sensitivity of heart fibres could account for the improved responsiveness of the hearts of physically trained animals to catecholamines [21] since training increased the phosphorylation level of the P-light chain [16] without altering the properties of β adrenergic receptors [22].

The determination of the phosphorylation level of the P-light chain by densitometric evaluation of the light chains after two-dimensional gel electrophoresis of skinned heart fibres is complicated by the fact, that the spot containing the phosphorylated light chain consists of 2 proteins: a non-phosphorylated and the phosphorylated form of the P-light chain referred to as LC-2* and LC-2-P, respectively, by [12,13]. This spot has been referred to as P-LC-2 in this article.

As the myosin light chain kinase used was purified to homogeneity [10] and as it is highly specific for the P-light chain [1,10], an increase in the relation P-LC-2 to LC-2 by MLCK treatment can only mean an increase in phosphorylated LC-2.

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