CYCLIC AMP-DEPENDENT INHIBITION OF SMOOTH MUSCLE ACTOMYOSIN

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1. Introduction

Injection of cAMP into smooth muscle fibres causes relaxation [1]. cAMP has been implicated to cause smooth muscle relaxation by stimulating the Ca-removing membrane system [2] or by activating the Na/K ATPase [3]. Both mechanisms might reduce the sarcoplasmic concentration of Ca^{2+} activator.

The mechanism of Ca^{2+} activation of smooth muscle myosin [4] is still controversial [5]. There is, however, much evidence that Ca^{2+} activate vascular smooth muscle actomyosin [6,7] and chicken gizzard actomyosin [8–10] via myosin phosphorylation by means of a Ca^{2+} and CDR-dependent myosin-light chain kinase. Relaxation and inhibition of smooth muscle actomyosin may be induced by dephosphorylation of the myosin light chain by means of a myosin-light chain phosphatase when the light chain kinase is inhibited. This inhibition may be brought about by removal of the activator Ca^{2+}. Alternatively inhibition of light chain kinase activity may, however, also be induced at constant free Ca^{2+} concentration by means of a cAMP and protein kinase-dependent phosphorylation of the light chain kinase [11]. Hence the actomyosin contractile system of smooth muscle ought to be inhibited by cAMP and cAMP-dependent kinase. We have investigated whether this is so.

2. Materials and methods

Actomyosin was extracted overnight at 5°C from hog carotid arteries at low ionic strength (80 mM NaCl, 4 mM EGTA, 20 mM imidazole (pH 7.6), 5 mM ATP) according to [4]. Crude actomyosin was precipitated by dialysis against ATP-free extraction solution, washed with this solution containing 1% Triton X-100 and then with Triton-free solution. It was then suspended (for 15 min at room temperature) either in ATPase buffer mixture containing (30 mM KCl, 10 mM $MgCl_2$, 10 mM imidazole, 0.1 mM EGTA) + 2 mM ATP and 20 mM NaF or alternatively in buffer mixture containing additionally 0.1 mM cAMP and 0.1 mM theophylline. In some cases (cf. table 1) the incubation mixtures were complemented by the addition of Sigma rabbit-muscle cAMP-dependent protein kinase (10 μg/ml). Phosphate incorporation into protein was measured by incubating the crude actomyosin as described, but replacing ATP by 2 mM [γ-^{32}P]ATP. The reaction was terminated by precipitating the protein with trichloracetic acid and subjected to gel electrophoresis for autoradiography as in [6]. The ATPase assay mixtures contained actomyosin suspended in 30 mM KCl, 10 mM imidazole, 2 mM Ca EGTA buffer which was sometimes (cf. table 1) complemented by the addition of 20 mM NaF or NaF and 0.1 mM cAMP and 0.1 mM theophylline (pH 7.2, T = 22°C). Reaction was started by the addition of ATP (2 mM) and terminated at regular time intervals by the addition of trichloracetic acid. ATPase was estimated from the linear portion of the time progress-curve of phosphate liberation as in [12], and protein was estimated using the Biuret method. Actomyocin super-precipitation was determined by measuring the super-precipitation (turbidity change) at 550 nm following the addition of ATP to the actomyosin suspended in ATPase buffer mixture. Chemically skinned fibre-bundles of Taenia coli were prepared as in [13] by means of the Triton extraction procedure and subsequently stored in a mixture (1:1) of glycerol and 'relaxing solution' (cf. legend fig.2) for 1–30 h. at −15°C. They were then fixed to a tension

recording device [14] and suspended in the solutions specified in fig.2.

3. Results and discussion

3.1. *Cyclic AMP induced actomyosin inhibition*

As shown [4] an increase in Ca^{2+} from 10^{-7}–10^{-5} M causes a pronounced activation of the Mg-dependent arterial actomyosin ATPase activity (fig.1a). This ATPase activity is depressed by ~35% after preincubation of the actomyosin with ATP and cAMP-dependent protein kinase. In these experiments 20 mM NaF was also added in order to inhibit endogenous protein phosphatase and 0.1 mM theophylline was added to inhibit cyclic nucleotide phosphodiesterase possibly contaminating the crude actomyosin preparation. The cAMP-induced inhibition is on average ~40% (cf. table 1) and hence much smaller than the ATPase inhibition caused by removing Ca^{2+} by EGTA (60–95% inhibition).

Figure 1a shows furthermore that the Ca^{2+} dependence of arterial actomyosin seems to be altered after preincubation of actomyosin with cAMP and cAMP-dependent protein kinase: 0.9 μM Ca^{2+} causes half maximum activation of the ATPase of 'natural' actomyosin, whereas it causes only 25% activation after incubation of the actomyosin with ATP, cAMP and cAMP-dependent protein kinase. Hence cAMP may be said to cause a 'rightward shift' of the curve relating activity and Ca^{2+} concentration by reducing the calcium sensitivity of crude arterial actomyosin. That is, treatment with cAMP causes a larger inhibition (> 60%) at half-maximum Ca^{2+} activation than under conditions of maximum Ca^{2+} activation. This inhibition of actomyosin ATPase after cAMP treatment of actomyosin is also associated with an inhibition in the rate of super-precipitation of actomyosin gels as shown in fig.1b.

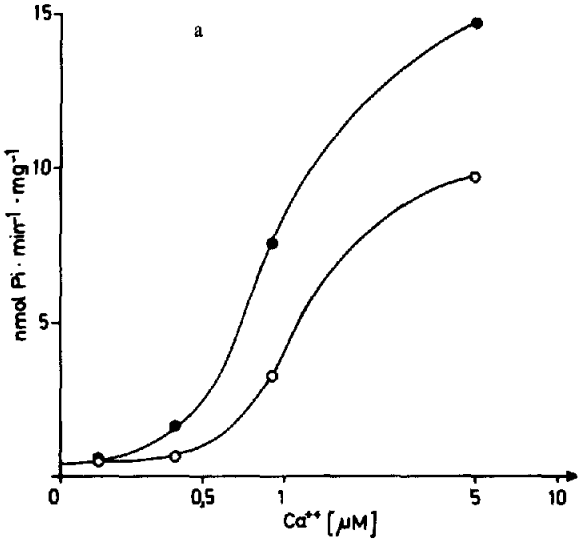

Fig.1a. Ca^{2+} dependence of arterial actomyosin ATPase activity (ordinate) before (•—•) and after cAMP-dependent phosphorylation (○—○): Conditions, cf. table 1, preparation II.

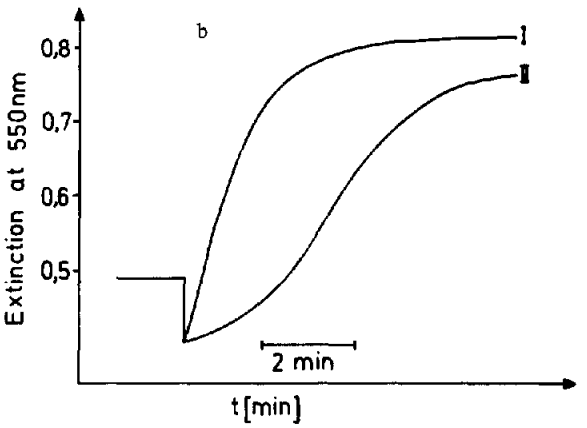

Fig.1b. Inhibition of arterial actomyosin 'supercontraction' by cAMP. Superprecipitation response measured as turbidity increase (A_{550}) following the addition of ATP (2 mM) before (I) and after (II) cAMP-dependent phosphorylation: conditions as in fig.1a.

In the above experiments cAMP was added in conjunction with a cAMP-dependent kinase. As shown in table 1 cAMP may, however, cause inhibition of crude arterial actomyosin by 40% (10–60%) even without the addition of exogenous kinase. This may suggest the presence of a contaminating cAMP-dependent endogenous protein kinase in crude arterial actomyosin. Preliminary experiments showed indeed that the cAMP-induced inhibition could not be observed after addition of the inhibitor of cAMP-dependent protein kinase (Sigma 1). The ATPase activity of purified actomyosin was not inhibited by cAMP (1 mM), theophylline (0.5 mM) and NaF (20 mM).

In the experiment in fig.2 we have investigated

Table 1
Inhibition of actomyosin ATPase activity by cAMP treatment

Actomyosin preparation	ATPase activity at 10^{-5} M Ca^{2+} (nmol . min^{-1} . mg $protein^{-1}$)	
	10^{-5} M Ca^{2+}	10^{-5} M Ca^{2+} + 0.1 mM cAMP
I	7.2	4.45
II	15.2	9.8
III	7.1	4.0
IV	16.6	8.3
V	7.5	6.9
VI	10	5.2

I, Mg-dependent ATPase activity determined in presence and absence of 0.1 mM cAMP and 0.1 mM theophylline (20 mM NaF also present in assays); II, ATPase activity of actomyosin preincubated for 15 min in ATPase buffer mixture containing 20 mM NaF, 2 mM ATP, 0.5 mM EGTA, 10 μg/ml bovine heart cAMP-dependent protein kinase (Sigma 1), 0.1 mM theophylline and either no cAMP or 0.1 mM cAMP. Preincubated actomyosin suspended in cAMP free ATPase buffer for assay; III–VI, Preparation pretreated as preparation II but without the addition of exogenous cAMP-dependent kinase

whether cAMP may also inhibit the Ca^{2+}-induced contraction of chemically skinned smooth muscle fibres suspended in ATP salt solution. Note that addition of 10 mM NaF to the bathing solution causes a slow relaxation, whereas the further addition of 0.5 mM cAMP and 0.5 mM theophylline enhances the rate and extent of relaxation. These effects can be reversed, after washing out NaF and cAMP. In some experiments the effect of NaF was more and that of cAMP less pronounced. The inhibition of contraction after prolonged incubation in presence of NaF and cAMP amounts to > 50% whereas a nearly complete inhibition can be achieved after reduction of Ca^{2+} to 10^{-6} M by means of EGTA. Note that the inhibiting effect of cAMP occurs at a constant concentration of ionized calcium since the latter is effectively buffered with 5 mM Ca–EGTA buffer and Ca-binding membranes had been destroyed by pre-extraction in solutions containing the non-ionic detergent Triton X.

3.2. *Possible mechanism of cAMP action*

It was found [11] that cAMP-dependent phosphorylation of chicken gizzard myosin light chain kinase causes an inhibition of this enzyme. This might account for the cAMP-induced inhibition of smooth muscle actomyosin ATPase and contraction of smooth muscle skinned fibres, described here for the first time, for conditions where Ca^{2+} concentration is held constant. The reversibility of this inhibition after washing out cAMP and fluoride might possibly be due to dephosphorylation of the light chain kinase by a protein phosphatase. An inhibition of smooth muscle actomyosin ATPase due to a cAMP-induced phosphorylation of the myosin light chain kinase has recently been shown (J. DiSalvo, personal communication).

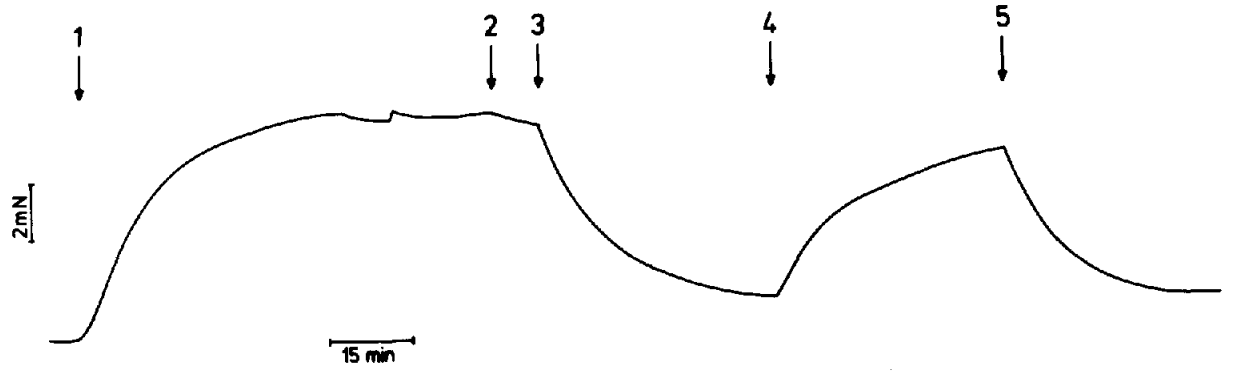

Fig.2. Inhibition of contraction of chemically skinned smooth muscle fibres by cAMP (Taenia coli or guinea pig) (1). Fibre bundle relaxed in ATP salt solution (containing 5 mM EGTA develops force after increasing Ca^{2+} to 10^{-5} M. (2) Partial relaxation after addition of NaF (10 mM). (3) Further relaxation induced by cAMP (0.5 mM) in presence of 0.5 mM theophylline. (4) Redevelopment of force after wash out of fluoride, cAMP and theophylline. (5) Complete relaxation after reducing Ca^{2+} to 10^{-8} M.

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