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Diverse actions of cadmium on the smooth muscle myosin phosphorylation system

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The effects of cadmium (Cd) on smooth muscle myosin phosphorylation have been investigated using an in vitro system comprising myosin filaments containing endogenous calmodulin (CM) and myosin light chain kinase (MLCKase). In the absence of calcium (Ca), Cd as well as some other divalent cations caused no activation of phosphorylation. However, when at least one (or possibly two) Ca^{2+} were bound per CM, the addition of 10 μM to 40 μM Cd^{2+} resulted in a 2 to 3 fold acceleration of the phosphorylation rate. Higher Cd concentrations caused inhibition of the system independent of Ca^{2+} concentration through the formation of Cd-ATP complexes. These results explain some previously controversial data on the complex effects of Cd in intact smooth muscles.

Myosin light chain phosphorylation; Calmodulin-Ca^{2+} complex; Cadmium; Smooth muscle

1. INTRODUCTION

For over a century it has been known that Cd intoxication is implicated in several diseases. More recently, it has been suggested that chronic subtoxic exposure to cadmium plays a role in some cardiovascular dysfunctions [1]. The mechanism of Cd action on smooth muscles is as yet poorly understood. Since smooth muscles exhibit a high degree of sensitivity to agents which inhibit Ca influx [2,3] and cadmium is a Ca channel blocker in cardiac cells [4], it may be expected that Cd influences smooth muscle activity via a similar route. However, current data indicate that the in vitro effects of Cd on smooth muscle tissues are highly concentration-dependent. High concentrations of Cd cause a dose-dependent inhibition of agonist induced contractions [5–7]. In contrast, Cd at low concentration can enhance the reaction of vascular smooth muscle to different agonists [8,9]. Such observations suggest that the effect of Cd ions on smooth muscle contractions is complex and may involve Ca channel blockage [10] as well as other processes.

The similarity of Cd and Ca ions suggests that cadmium can interact with Ca^{2+}-binding sites and in this way disturb the processes which, physiologically, are regulated by calcium. One potential target for cadmium, found in all eukaryotic cells is the metal binding protein, calmodulin (CM), which contains four Ca^{2+}-binding domains. Cadmium can occupy these four Ca^{2+}-binding sites causing similar CM conformational changes to those induced by Ca^{2+} [11]. In smooth muscles, Ca_4^{2+}-CM (and possibly Ca_3^{2+}-CM) complexes activate MLCKase [12]. It is still controversial whether replacement of Ca^{2+} by Cd^{2+} in these complexes produces similar active states.

We therefore found it necessary to reexamine the activation of MLCKase by Cd and other divalent cations. The results obtained, using a filamentous myosin system that may be taken to closely resemble the in vivo situation, appear to explain some of the controversial observations described previously.

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2. MATERIALS AND METHODS

Myosin phosphorylation assays were carried out in a buffer containing (in mM): 60 KCl, 2 $MgCl_2$, 0.5 DTE, 20 Tris. In our experiments EGTA could not be used to buffer free Ca^{2+} concentration because of its high affinity for Cd^{2+} (about 10^4 that for Ca^{2+}). Calcium was therefore removed by storing all buffers in plastic containers over Chelex-100 (Bio-Rad, Richmond, USA) resin. The resin was prepared as described by the manufacturer. Before use the pH of the buffers were adjusted to 7.2 with HCl.

A filamentous myosin system containing endogenous calmodulin (CM) and myosin light chain kinase (MLCKinase) was prepared from turkey gizzard as previously described [13]. It was established earlier [14] that the molar ratio of CM and MLCKase to myosin in such preparations is below 1:100, the CM to kinase ratio being approximately stoichiometric.

The system (suspension of myosin filaments) was freed of Ca^{2+} ions by the addition of EGTA at a final concentration 0.1 mM. After pelleting by centrifugation, the supernatant was removed and the pellet was resuspended in the Ca^{2+}-free buffer described above. The procedure was repeated twice.

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The influence of divalent cations on phosphorylation was investigated in two different sets of experiments: In the first set the cations were added to the myosin system freed of Ca. In the second set calcium was added to the Ca^{2+}-free myosin system to give the required final concentration and the effects of the other cations were investigated. Phosphorylation assays were carried out as described previously [13]. Curves illustrating the phosphorylation dependence on the cation concentration were obtained from a number of separate incubations containing different cation concentrations.

3. RESULTS

Addition of ATP to filamentous myosin that had not been rinsed with EGTA but only resuspended in Ca-free solution resulted in phosphorylation, indicating that Ca was still present in the system prepared in this way. Concentrations of Cd up to 4×10^{-6} M had no influence on the myosin phosphorylation under these conditions. In the concentration range 2×10^{-5} M–10^{-3} M Cd caused a gradual inhibition of the phosphorylation. Under the same conditions Ba caused inhibition of phosphorylation at concentrations higher than 10^{-4} M (Fig. 1). A gradual inhibition was also observed when the Ca concentration exceeded 10^{-4} M (see Fig. 2, high Ca concentration range).

After rinsing filamentous myosin with the EGTA containing buffer the concentration of Ca was reduced sufficiently to prevent measurable phosphorylation in the Ca-free buffer. Addition of Ca^{2+} to the system then restored the reaction in a concentration dependent manner as expected (Fig. 2). Significantly, under similar conditions Ba, Cd and Co did not induce phosphorylation (Fig. 3). Sr caused phosphorylation which did not exceed 30% of that induced by Ca.

The most interesting effects were seen at unsaturated levels of Ca^{2+}. As demonstrated in Fig. 4, at 20 μM of Ca, increasing amounts of Cd, Co and Sr (but not Ba),

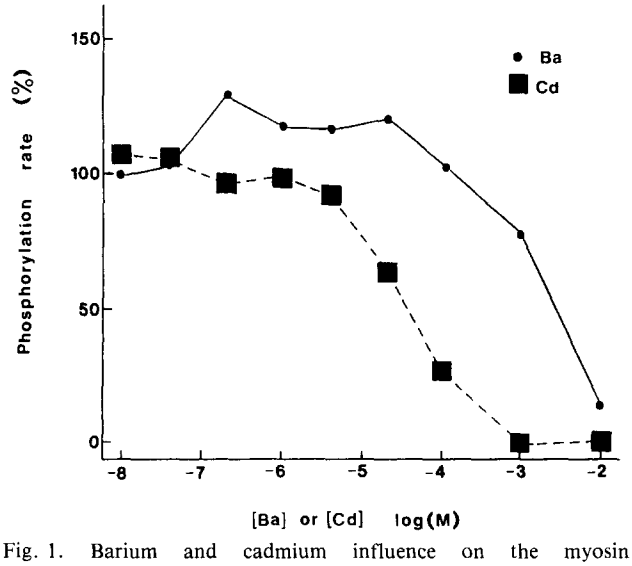

Fig. 1. Barium and cadmium influence on the myosin phosphorylation. The filamentous myosin system untreated with EGTA. The phosphorylation rate with no cation added was set as 100%.

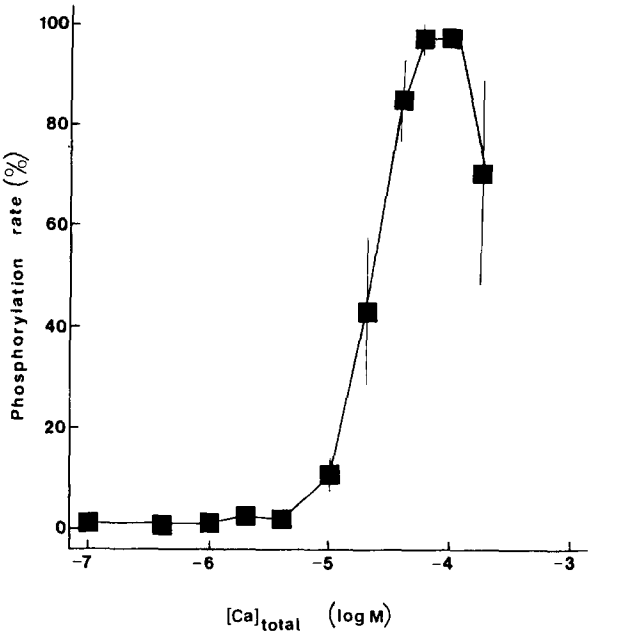

Fig. 2. The concentration-phosphorylation curve for Ca on the filamentous myosin system washed with 0.1 mM EGTA. Maximal phosphorylation rate was set as 100%.

at concentrations 20 μM–240 μM, caused an increase of phosphorylation. However, when the Ca concentration in the system equaled 100 μM, Cd at concentrations higher than 40 μM gradually inhibited the phosphorylation. Such inhibition was not observed for the other cations (Fig. 5).

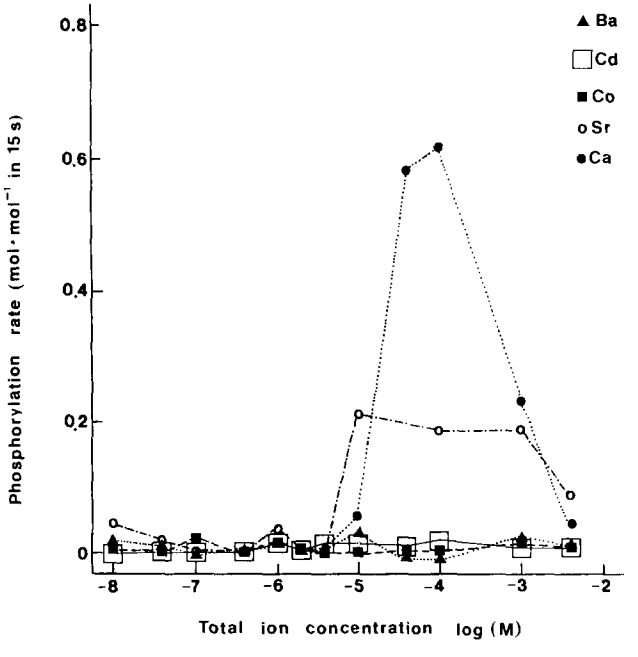

Fig. 3. The influence of Ca, Ba, Co, and Sr on the myosin phosphorylation in the filamentous myosin system washed with 0.1 mM EGTA.

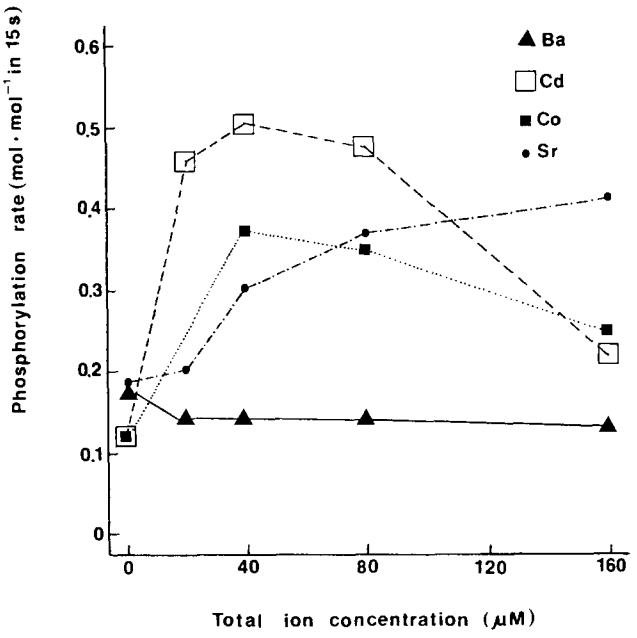

Fig. 4. The effects of Ba, Cd, Co, and Sr on the myosin phosphorylation in the presence of 20 μM Ca.

Fig. 6 shows the myosin phosphorylation as a function of Cd^{2+} concentration for different concentrations of Ca^{2+}. In the presence of Ca^{2+}, the response to increasing concentration of Cd^{2+} was biphasic. The initial gradual increase of the phosphorylation to a certain maximum was followed by its inhibition. The elevation of Ca caused a decrease of the peak phosphorylation and shifted it towards the lower concentrations of cadmium.

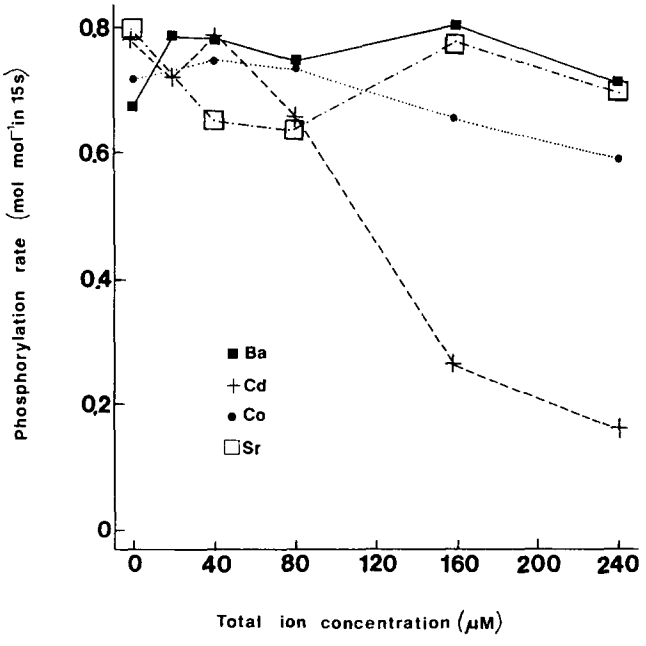

Fig. 5. The effects of Ba, Cd, Co, and Sr on the myosin phosphorylation in the presence of 100 μM Ca.

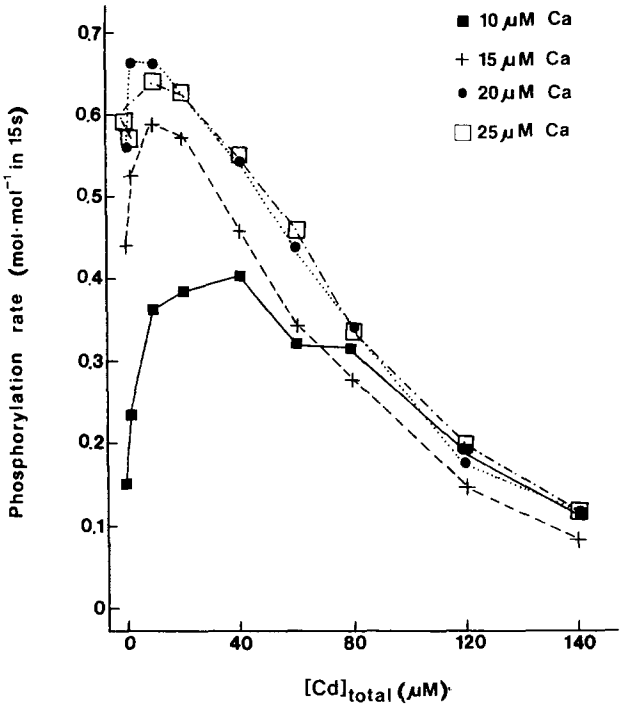

Fig. 6. The influence of Cd on the myosin phosphorylation in the presence of different concentrations of Ca.

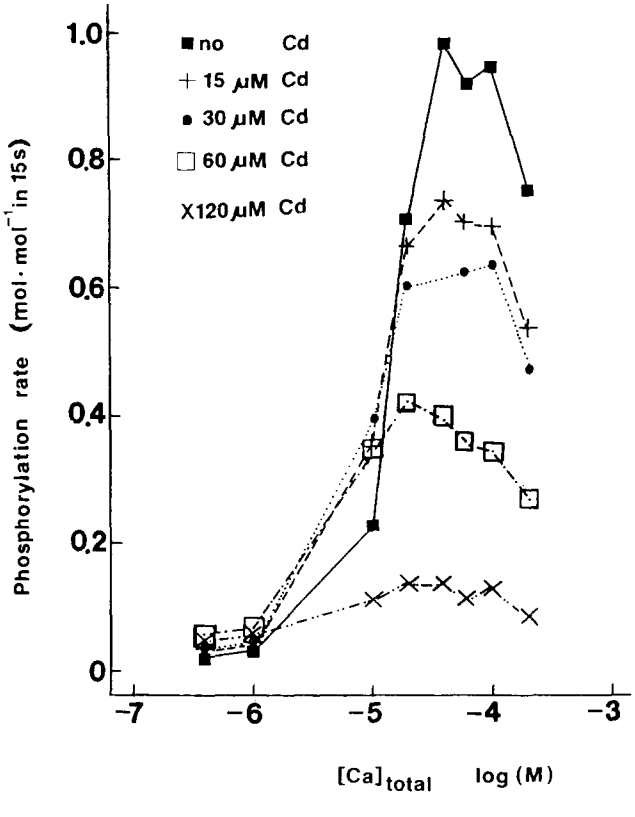

Fig. 7. The effects of different concentrations of Cd on the concentration-phosphorylation curves for Ca.

A dual effect of cadmium on the dependence of myosin phosphorylation on calcium was most clearly demonstrated when the Ca concentration was varied within the whole activation range (0.5 μM–100 μM) (Fig. 7). In this case Cd (15–120 μM) was added prior to Ca administration. An increasing Cd concentration resulted in a gradual lowering of maximal phosphorylation. Simultaneously, the presence of Cd (except 120 μM) enhanced phosphorylation caused by concentrations of Ca lower than 2×10^{-5} M. Thus, as before, a partial saturation of CM by Ca^{2+} was required for the activatory effect of Cd^{2+} ions.

4. DISCUSSION

It has been reported that Ca^{2+} binding to CM is not specific [15] and cations differ in their ability to induce conformational changes in CM. Chao et al. [16] in their study on phosphodiesterase have demonstrated that Cd and Sr stimulated CM-dependent activity of this enzyme while Co and Ba were essentially ineffective. It has also been recently concluded that Cd induces similar conformational changes of CM as Ca [11].

In contrast to these results, our experiments on the Ca^{2+}-free filamentous myosin system indicate that Cd is unable to substitute Ca in the activation of myosin phosphorylation. Of the other cations tested, only Sr stimulated myosin phosphorylation without Ca. This discrepancy is possibly explained by greater specificity of the filamentous myosin system that may retain a more native form of the enzyme-substrate complex.

The data presented here, however, indicate that while cadmium alone does not stimulate myosin phosphorylation, it can influence the phosphorylation caused by calcium. The effect caused by Cd was dependent on both the Cd as well as the Ca concentration.

One possible explanation of the observed effects of Cd on the myosin phosphorylation is that Cd can cause activation of MLCKinase in such cases when at least one or maybe two Ca^{2+} ions are bound per molecule of CM. The Cd_4^{2+}-CM complex appears to be unable to activate MLCKinase. A stimulation of MLCKinase resulting from increased free Ca^{2+} caused by Cd displacement is less probable since under the conditions used in our experiments the concentration of CM was in the range of 50–200 nM. Thus, release of Ca^{2+} from CM upon Cd binding, could not increase the Ca^{2+} level by observed 20–40 μM. Myosin as a major protein component of the system could also be considered to produce a similar Ca pool. However we consider this to be unlikely, since its affinity for Ca is considerably lower than that of CM [14]. The inhibition of the myosin phosphorylation by Cd at concentrations of about 10^{-4} M is, at least partially, caused by formation of Cd-ATP complexes in agreement with the 4×10^4 M^{-1} apparent binding constant of Cd for ATP [17].

A biphasic influence of Cd on MLCKinase activity has been observed also by Mazzei et al. [18]. However, we did not observe any Cd-induced activation of MLCKase in the Ca^{2+}-free system. This difference may be related to the fact that 50 μM of EGTA, used by these authors, could be insufficient to remove all contaminating calcium. This interpretation is consistent with our observation of a pronounced Cd^{2+} activation occurring at low (10–20 μM) Ca^{2+} concentration. The addition of Cd to the medium containing Ca-EGTA complexes can also cause an increase of free Ca^{2+} as has been discussed by Verbost et al. [19].

Studies in vitro showed that in the presence of external Ca (1.5 mM–2 mM), Cd at concentrations higher than 10^{-6} M decreased isometric contractions of smooth muscles [18,20]. In Ca^{2+}-free conditions, however, 10^{-5} M Cd contracted strips of human uterine arteries [20]. The data obtained here suggest that the mechanism of these 'Ca-free contractions' may involve the influence of Cd on the myosin phosphorylation system.

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REFERENCES

[1] Perry, H.M., Erlanger, M. and Perry, E.F. (1979) Environm. Health Persp. 28, 251–260.
[2] Fleckenstein, A. (1985) Med. Res. Rev. 5, 395–425.
[3] Kostrzewska, A., Laudański, T. and Batra, S. (1988) Br. J. Pharmacol. 94, 1037–1042.
[4] Lee, K.S., Lee, E.W. and Tsien (1982) Biophys. J. 37, 342a.
[5] Thind, G.S., Stephan, K.F. and Blakmore, W.S. (1970) Am. J. Physiol. 219, 577–583.
[6] Thoda, N. (1975) Am. J. Physiol. 225, 350–355.
[7] Hayashi, S. and Toda, N. (1977) Br. J. Pharmac. 60, 35–43.
[8] Nechay, B.R. (1978) J. Toxicol. Environ. Health 4, 557–567.
[9] Niwa, A. and Suzuki, A. (1982) J. Toxicol. Sci. 7, 51–60.
[10] Hurwitz, L. (1986) Annu. Rev. Pharmacol. Toxicol. 26, 225–258.
[11] Sutoo, D., Akiyama, K., Fujii, N. and Matsushita, K. (1986) Jpn. J. Pharmacol. 40, 169–173.
[12] Mamar-Bachi, A. and Cox, J.A. (1987) Cell Calcium 8, 473–482.
[13] Sobieszek, A. (1985) Biochemistry 24, 1266–1274.
[14] Sobieszek, A. (1990) J. Muscle Res. Cell Motil., in press.
[15] Cheung, W.Y. (1985) Harvey Lectures, ser. 79, 173–216.
[16] Chao, S.H., Suzuki, Y., Zysk, J.R. and Cheung, W.Y. (1984) Mol. Pharmacol. 26, 75–82.
[17] Stephenson, D.G. and Thieleczek, R. (1986) J. Physiol. 380, 75–92.
[18] Mazzei, G.J., Girard, P.R. and Kuo, J.F. (1984) FEBS Lett. 173, 124–128.
[10] Verbost, P.M., Flik, G., Lock, R.A.C. and Wendelaar Bonga, S.E. (1988) J. Membr. Biol. 102, 97–104.
[20] Huang, Y., Quayle, J.M., Worley, J.F., Standen, N.B. and Nelson, M.T. (1989) Biophys. J. 56, 1023–1028.