ACTIVE EXTRUSION OF CALCIUM IONS BY SMOOTH MUSCLE MICROSOMES

Masaatsu Uchida
Department of Molecular Pharmacology
Meiji College of Pharmacy
1-35 Nozawa, Setagayaku
Tokyo, 154, Japan

Received August 11,1976

ABSTRACT Microsomes from intestinal and aortic smooth muscles were loaded with Ca ions at 0°C for 4 hrs, then the medium was diluted with Ca(-), or Ca(+)-solution and the time course of Ca ion flux with or without ATP was tested by Millipore filtration. It was found that ATP caused release of Ca from intestinal microsomes and uptake of Ca by aortic microsomes. Marker enzymes showed that the intestinal microsomes were mainly composed of plasma membranes and the aortic microsomes of the sarcoplasmic reticulum. Thus the active release of Ca from the intestinal microsomes was associated with plasma membranes. The active release mechanism is probably responsible for efflux of Ca from muscle strips during relaxation.

INTRODUCTION

Ca ions are closely related to muscular activities. From numerous studies on various types of smooth muscles it is now thought that the cytoplasmic level of free Ca is regulated by two mechanisms (1) : sequestration of Ca into intracellular locations, such as the sarcoplasmic reticulum and mitochondria and extrusion of Ca into the extracellular fluid through the plasma membrane. *In vitro* experiments on Ca uptake by various microsomal fractions have shown that Ca ion sequestration depends on ATP (2-9), whereas efflux of Ca ions from the muscle strips preloaded with radioactive Ca ions was observed during relaxation suggesting that it may also be correlated with relaxation mechanisms (10-14). In this work the efflux of Ca from microsomal preparations from aortic and intestinal smooth muscles was studied *in vitro* under conditions where efflux could be distinguished from sequestration.

Materials and Methods

Preparation of microsomal fractions

Aortic microsomes were obtained from rabbits as described by Fitzpatrick *et*

al.(6). Intestinal microsomes were obtained from cat small intestinal smooth muscles as described previously (15). The microsomal fractions obtained were suspended by flocculation in ice-cold 0.32 M sucrose solution containing 0.3 mM $CaCl_2$ (approx. 5 μCi ^{45}Ca/ml) and 2 mM $MgCl_2$ and adjusted to pH 7.2 with imidazole at a protein concentration of 3-6 mg/ml, determined by the micro-biuret method (16). The suspension was stored at 0°C. The radioactivity of the microsome increased with time and reached a plateau within 4 hrs. The time course of increase was similar to that observed on loading synaptosomes with Ca (17). After 4 hrs, the suspension was centrifuged at 10,000 x g for 20 min using an ice-cold titanium roter and the supernatant was used for experiments. As marker enzymes, activities of 5'-nucleotidase and NADH cytochrome c reductase were examined (9,18).

Experiment 1

The supernatant was diluted 20-fold with Ca-free 50 mM imidazole buffer (pH 7.2) containing 2 mM $MgCl_2$, 100 mM KCl and 5 mM potassium azide at 22°C. Samples were taken after various times and filtered through Millipore filters (HA 0.45 μm) and the radioactivity of the filters was counted by a liquid scintillation spectrometer (9). In some cases 3 mM ATP (Tris salt) was added alone or in the presence of 3 mM iodoacetic acid. (Concentration of drugs are given as final concentration in this paper.)

Experiment 2

Conditions were as for Experiment 1 except that the buffer contained 0.3 mM $CaCl_2$ (nonradioactive).

Results

The aortic microsomal preparation was rich in NADH cytochrome c reductase (rotenone insensitive), which is a marker of endoplasmic reticulum, whereas that of intestinal microsomes was rich in 5'-nucleotidase, a marker of the plasma membrane. These findings are in accordance with those of Hurwitz *et al.* (6). By electron microscopy the preparations were seen to consist of smooth surfaced, closed vesicles of 0.3-0.8 μm diameter (19). They had the same diameter as the microsacs of electric organs, which have been used for the study of ion flux *in vitro* (20,21).

In Experiment 1, after equilibration of the microsomes with the medium, the suspension was diluted with Ca-free medium. These experimental conditions caused passive efflux of loaded Ca ions. The effect of ATP on this Ca efflux was tested. Figure 1-A shows results with intestinal microsomes. ATP accelerated Ca ion flux and its effect was blocked by iodoacetic acid. Figure 1-B shows results with aortic microsomes. Addition of ATP resulted in uptake of Ca ions and this was inhibited by iodoacetic acid.

In Experiment 2, the calcium concentration was not diluted and under equi-

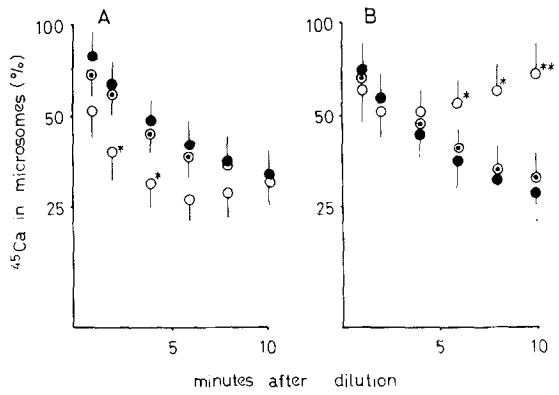

Figure 1. Experiment 1

Suspensions of microsomes loaded with ^{45}Ca were diluted with Ca-free solution and the time course of the change in ^{45}Ca content of the microsomes was examined. A; intestinal microsomes, B; aortic microsomes. ●; control, ○; + ATP (3 mM), ◎; + ATP + iodoacetic acid (3 mM). Ca contents of the microsomes are expressed as percentages of the value at the equilibrium after dilution which was calculated according to Kasai and Changeux (20). Vertical bars represent standard errors of 5 determinations. The statistical significance with respect to the control was evaluated by Student's t-test.

* ; $p<0.05$, ** ; $p<0.01$

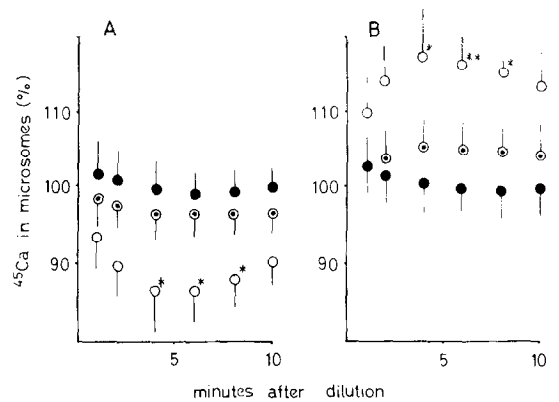

Figure 2. Experiment 2

Experimental conditions were as for Experiment 1 except that the diluting solution contained 0.3 mM $CaCl_2$ (nonradioactive). Ca contents were expressed as percentages of the value at the equilibrium after dilution which was the average value of control samples at various times. Other notes are the same as in Figure 1.

librium of the Ca ions inside and outside the microsomes ATP was added to the suspension. As shown in Figure 2-A, microsomes from small intestinal muscles

released Ca ions in the presence of ATP, whereas in aortic microsomes Ca ions were sequestered by ATP as shown in Figure 2-B. The effects of ATP on the two kinds of muscle microsomes both inhibited by iodoacetic acid.

Discussion

Previous workers have shown that smooth muscles differ in sensitivity to Ca depletion (6,22-24). The main pulmonary artery of the rabbit when immersed in Ca-free medium retains its capacity to contract long after extracellular Ca ions have been removed, whereas under the same conditions the intestinal muscles only retain their capacity to contract for a short time. The difference in the durations of contractile responses of different types of smooth muscles in Ca-depleted medium may be related to differences in the volumes of their Ca storage structures (7) or to differences in the affinities of their storage sites for Ca (1). Plasma membarnes of various cells have the ability to transport ions against a concentration gradient and plasma membranes of smooth muscles are important in extrusion of Ca ions from tissues (25,26). This Ca extrusion may depend on ATP (27) or on a Na-Ca exchange system (28). In taenia coli of guinea pig the latter mechanism has been excluded (26). Hurwitz *et al.* (7) showed that Ca uptake activity of microsomes from intestinal smooth muscle of guinea pig is mainly associated with the plasma membranes, whereas that of rabbit aortic smooth muscle is mainly associated with intracellular structures.

Unlike in previous works, in the present study the membrane vesicles were loaded with Ca ions before adding ATP. Active extrusion of Ca by intestinal microsomes can be attributed to the activity of plasma membrane vesicles and Ca uptake by aortic microsomes to fragmented sarcoplasmic reticulum. Two different mechanisms, efflux and sequestration regulate the intracellular Ca level and both these mechanisms operate in both muscles, so the apparent Ca ion movements which were observed in this study depended on the balance between the activities of ion translocation by the two kinds of membrane struc-

tures. In intestinal microsomes ATP accelerated Ca efflux in Experiment 2 and this may be due to the same mechanism as the active efflux observed with muscle strips (10-14). On the other hand, in aortic microsomes ATP stimulated Ca sequestration. The role of sarcoplasmic reticulum in the aorta may be similar to that in skeletal muscles. However, although intestinal microsomes can take up Ca ions actively (1,7,9,29), active extrusion is probably more important in relaxation. Thus in regulating the level of Ca active extrusion through the plasma membrane may compensate for the relatively low sequestering activity of the sarcoplasmic reticulum. In contrast, in aortic muscle, the sarcoplasmic reticulum is more important in Ca regulation.

These conclusions from these experiments *in vitro* agree with physiological and histological evidence for the existence of a functional sarcoplasmic reticulum in vascular smooth muscle, for its relative insignificance in intestinal smooth muscle and for active Ca extrusion through the plasma membrane, which may mediate Ca efflux during relaxation.

References

1. McGuffee, L.T., and Bagby, R.M. (1976) Amer. J. Physiol. 230, 1217-1224.
2. Carsten, M.E. (1969) J. Gen. Physiol. 53, 414-426.
3. Batra, S.C., and Daniel, E.E. (1971) Comp. Biochem. Physiol. 38, 369-385.
4. Somlyo, A.V., and Somlyo, A.P. (1971) Science, 174, 955-958.
5. Anderson, R., and Nilson, K. (1972) Nature, New Biol. 238, 119-121.
6. Fitzpatrick, D.F., Landon, E.J., Debbas, G., and Hurwitz, L.A. (1972) Science, 176, 305-307.
7. Hurwitz, L., Fitzpatrick, D.F., Debbas, G., and Landon, E.S. (1973) Science, 179, 384-386.
8. Hess, M.L., and Ford, G.D. (1974) J. Mol. Cell. Cardiol. 6, 275-282.
9. Tomiyama, A., Takayanagi, I., and Takagi, K. (1975) Biochem. Pharmacol. 24, 9-12.
10. Goodford, P.J. (1965) J. Physiol. (London), 176, 180-193.
11. Nagasawa, J. (1965) Tohoku J. Exp. Med. 85, 72-83.
12. Karaki, H., Ikeda, M., and Urakawa, N. (1969) Jap. J. Pharmacol. 19, 291-300.
13. Goodford, P.J., and Wolowyk, M.W. (1972) J. Physiol. (London) 224, 521-535.
14. Tomiyama, A., Takayanagi, I., and Takagi, K. (1973) J. Pharm. Pharmacol. 25, 65-68.
15. Uchida, M., and Takagi, K. (1973) Jap. J. Pharmacol. 23, 337-347.
16. Itzhaki, R.F., and Gill, D.M. (1964) Anal. Biochem. 9, 401-412.
17. Ichida, S., Hata, F., Matsuda, T., and Yoshida, H. (1976) Jap. J. Pharmacol. 26, 31-38.
18. Luisier, A.L., Robinson, J.W.L., and Kinne, R. (1974) Experientia, 30, 631-633.

19. Uchida, M., and Takagi, K. (1976) Europ. J. Pharmacol. in press.
20. Cartaud, J., Benedetti, E.L., Kasai, M., and Changeux, J.P. (1971) J. Memb. Biol. 6, 81-108.
21. Kasai, M., and Changeux, J.P. (1971) J. Memb. Biol. 6 1-23.
22. Hurwitz, L., Joiners, P.D., von Hagen, S. (1969) Amer. J. Physiol. 216, 215-219.
23. Somlyo, A.P., Devine, C.E., Somlyo, A.V., and North, S.R. (1971) J. Cell. Biol. 51, 722-741.
24. Devine, C.E., Somlyo, A.V., and Somlyo, A.P. (1972) J. Cell. Biol. 52, 690-718.
25. Schatzmann, H.J. (1961) Pflügers Arch. 274, 295-306.
26. Casteels, R., and van Breemen, C. (1975) Pflügers Arch. 359, 197-207.
27. Schatzmann, H.J., and Vincenzi, F.F. (1969) J.Physiol. (London) 201, 369-395.
28. Blaustein, M.P., and Hodgkin, A.L. (1969) J. Physiol. (London) 200, 497-527.
29. Yamashita, H., Takayanagi, I., Manda, T., and Takagi, K. (1976) Japan. J. Pharmacol. in press.