Vol. 119, No. 2, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 15, 1984 Pages 652-656

POSSIBLE INVOLVEMENT OF ACTIN AND MYOSIN IN Ca^{2+} TRANSPORT THROUGH THE PLASMA MEMBRANE OF CHROMAFFIN CELLS

Orna E. Harish, Rina Levy, Kurt Rosenheck* and Avrahem Oplatka

Departments of Polymer and *Membrane Research
The Weizmann Institute of Science, Rehovot 76100, Israel

Received January 27, 1984

The exocytosis of catecholamines by chromaffin cells following stimulation (e.g. by acetylcholine) is accompanied by a rise in the level of intracellular free Ca^{2+}. Actually, secretion can be induced merely by making the cells leaky to Ca^{2+} from the external medium. We have recently demonstrated that secretion can be increased by the introduction of DNase-I, the F-actin depolymerizing agent, or of heavy meromyosin, the enzymatically active fragment of myosin. Suspecting that these changes might be associated with a higher intracellular level of Ca^{2+}, we now have measured the influx of $^{45}Ca^{2+}$ into chromaffin cells which have undergone fusion with DNase-I- or with heavy meromyosin-loaded liposomes. In both cases, a marked increase in Ca^{2+} uptake has been observed, which could be abolished by Co^{2+} ions (a Ca^{2+} channel blocker), suggesting an intimate involvement of the cellular actomyosin system in the process of Ca^{2+} ions transport through the Ca^{2+} channels of the plasma membrane.

Actin and myosin are believed to be responsible for a variety of motility phenomena in non-muscle cells. They have been found also in the catecholamine-secreting chromaffin cells and it has been suggested that they might be involved in the movement of the catecholamine-loaded chromaffin granules towards the plasma membrane and/or the fusion process which precedes exocytotic secretion (1,2). Actin filaments appear to be associated with the plasma membrane (3). We have recently demonstrated that secretion from adrenal medullary cells can be increased by introducing, with the aid of liposomes, DNase-I, the F-actin depolymerizing agent, or heavy meromyocin (HMM), the enzymatically active fragment of myosin (4,5). The exocytosis of catecholamines by chromaffin cells following stimulation by various secretagogues is dependent upon a rise in the intracellular level of free Ca^{2+} (6). We have therefore considered the possibility that actin filaments, with or without myosin, might play a role in the regulation of Ca^{2+} movements through the plasma membrane and have measured the uptake of $^{45}Ca^{2+}$ by chromaffin cells into which DNase-I or HMM had been introduced.

MATERIALS AND METHODS: Chromaffin cells were isolated from bovine adrenal medulla according to Schneider et al. (7). The proportion of chromaffin cells was 85-95%, the rest being cortical, endothelial and red blood cells.

Abbreviations: ACh - acetylcholine, HMM - heavy meromyosin.

0006-291X/84 $1.50

Liposome preparation: Small unilamellar vesicles, referred to as liposomes, were prepared according to the French Press method (8,9). Phosphatidylcholine, phosphatidylserine and cholesterol (molar ratio 7:1:2) were mixed in a chloroform-methanol (1:1) solution, and dried to a thin film with nitrogen gas. 2 ml of the aqueous solution to be entrapped were added, and the mixture was vortexed until all traces of phospholipids disappeared from the surface of the container. The resulting multi-lamellar vesicles were allowed to swell for an hour. This mixture was then put through a French Press four times, at 20,000 psi. Each time, the mixture was bled slowly from the cell of the French Press, drop by drop. The final suspension was centrifuged for 2 minutes in the microfuge, and dialyzed against Ca^{2+}-free Krebs-Ringer: (in mM) NaCl(118), KCl (4.7), KH_2PO_4(1.2), $MgSO_4$ (1.2), $NaHCO_3$ (25), D-glucose(10) [Buffer I].

Interaction of liposomes with chromaffin cells: 100λ of the liposomes were added to 1 ml of cell suspension ($1x10^6$ cells/ml) and incubated for 1 hr at 37°C. The cells were then washed with Buffer I supplemented with 2.2 mM $CaCl_2$ and 5 mg/ml BSA.

Ca^{2+} uptake: The cells were divided into two fractions and 0.5 mM acetylcholine (ACh) was added to one of them. 10λ Ca^{2+} (2 mg/ml, specific activity 12.3 mCi/mg) were immediately added to both fractions. Samples were taken at different times for the determination of $^{45}Ca^{2+}$ uptake by ion exchange chromatography (10). $^{45}Ca^{2+}$ taken up into the cells was measured in a liquid scintillation counter.

HMM was prepared according to Weeds and Pope (11). DNase-I was a Sigma product.

RESULTS AND DISCUSSION: In two other articles (Lelkes, Friedman, Oplatka and Rosenheck, submitted for publication) we have presented quantitative data as to the efficacy of liposomal delivery in the system studied in the present paper. Thus, microscopic visualization indicated that fluorescent macromolecules, including rhodamine-labelled G-actin, could be introduced into nearly all cells of a given population.

Figs. 1 and 2 represent the time-courses of the $^{45}Ca^{2+}$ uptake induced by DNase-I and HMM respectively, as compared to control cells treated with "buffer liposomes" containing only the medium in which the proteins had been dissolved. The time-courses of $^{45}Ca^{2+}$ uptake by the controls were identical with those obtained with cells to which no liposomes had been added. The increase in $^{45}Ca^{2+}$ uptake following stimulation with ACh with no protein incorporation is in line with work reported recently by Oka et al. (12) and Kilpatrick et al. (13). As can be seen from Figs. 1 and 2, incorporation of either DNase-I or HMM resulted in a significant increase in the rate of Ca^{2+} uptake for both stimulated and un-stimulated cells.

The increase in Ca^{2+} uptake following incubation with DNase-I could in principle be ascribed to permeabilization of the plasma membrane at a result of a depolymerizing effect on the plasma membrane-associated actin network which might serve as sort of mechanical "support" to the membrane. Transport of ions through such a "leaky" membrane should, however, be non-specific. A case in point are the "leaky" cells of Baker and Knight (6) obtained by application of high electric field pulses on chromaffin cells,

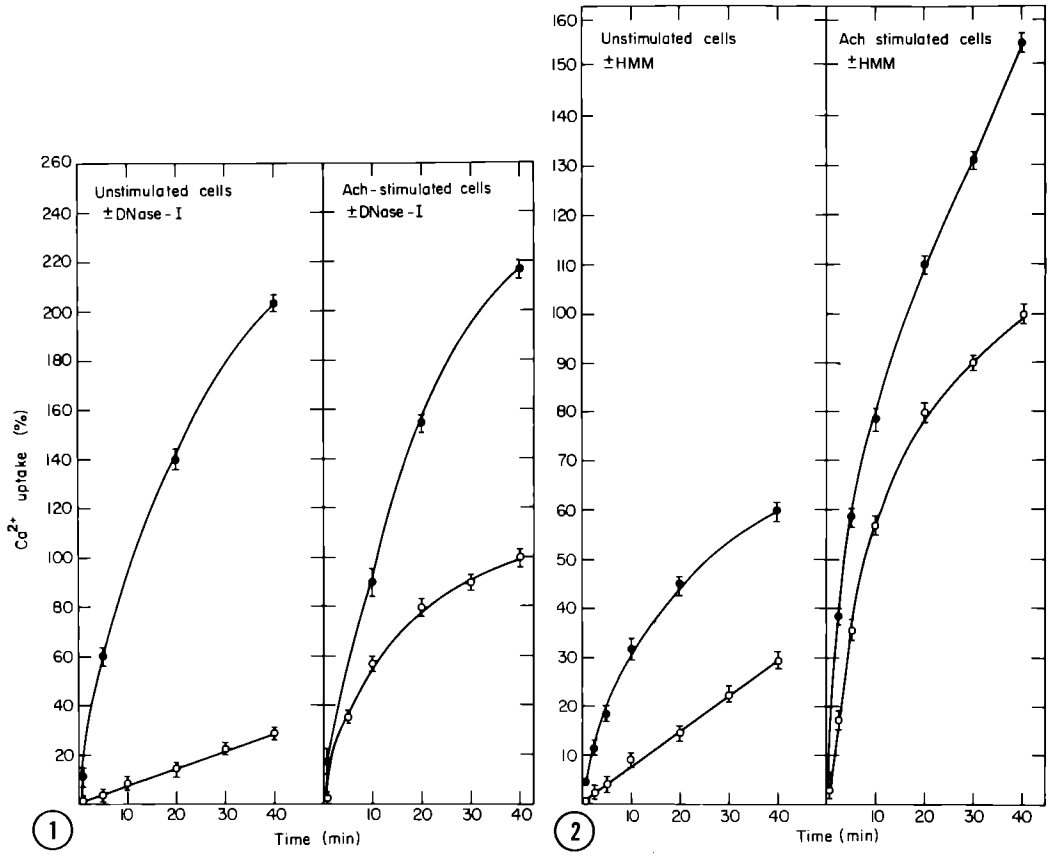

Fig. 1: Effect of DNase-I on Ca^{2+} uptake by chromaffin cells. 2 ml of a DNase-I solution (5 mg/ml in 130 mM KCl, 10 mM NaCl) were added to 16.5 mg of the phospholipid-cholesterol mixture (see text). The behaviour was practically the same when 0.1 mM of the antiprotease phenylmethylsulfonylchloride (PMSF) was also present in the DNase-I solution. The results are expressed in percent, 100% being the cpm obtained from cells stimulated by ACh in the absence of liposomes. 100% corresponds to about $3x10^3$ cpm. The results given in this figure are from one experiment (in triplicate) which is representative of 5 different experiments.
Open symbols - buffer liposomes
Closed symbols - DNase-I-loaded liposomes.

Fig. 2: Effect of HMM on Ca^{2+} uptake by chromaffin cells. 2 ml of an HMM solution (7 mg/ml in 130 mM KCl, 10 mM NaCl) were added to 16.5 mg of the phospholipid-cholesterol mixture. Other details as in the Legend to Fig. 1.
Open symbols - buffer liposomes
Closed symbols - HMM-loaded liposomes.

in which exocytosis of catecholamines induced by Ca^{2+} influx through the leaky plasma membrane cannot be inhibited by specific blockers of Ca^{2+} channels. This does not seem to be the case for the system studied by us, since application of Co^{2+} ions, a Ca^{2+}-channel blocker (14) abolished Ca^{2+} uptake by DNase-I-treated cells (Fig. 3).

The fact that HMM was found to give effects similar to those obtained with DNase-I with respect to Ca^{2+} uptake (as well as secretion and depola-

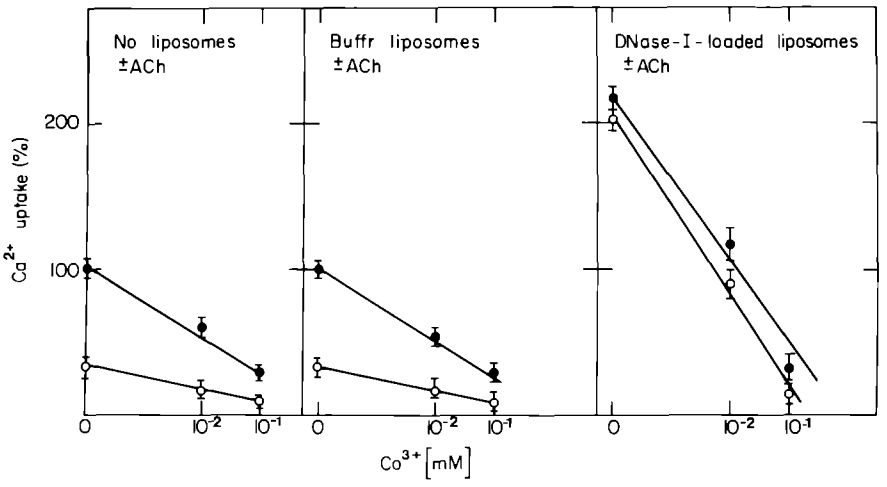

Fig. 3: Effect of Co^{2+} on Ca^{2+} uptake by chromaffin cells in the presence and absence of incorporated proteins.
$CoCl_2$ was added just before the addition of Ca^{2+}. $^{45}Ca^{2+}$ uptake was measured after 40 min. The experiment was done in triplicate and represents 3 different experiments.
Open symbols - no stimulation
Closed symbols - ACh stimulation.

rization (4,5) suggests the possibility that in the untreated cells, actin acts in conjunction with cellular myosin in modulating, directly or indirectly, the Ca^{2+} fluxes through their respective channels. As we have shown, HMM is mechanochemically competent, but less so than intact myosin (15). Therefore, partial replacement of the cellular myosin by HMM in the actomyosin complex may lead to a reduction in its effectiveness and thus to the enhanced Ca^{2+} uptake we have observed.

It is interesting to note that the secretory behavior of leaky cells which we have prepared according to Baker and Knight (6), and which can secrete catecholamines without any stimulation following the incorporation of Ca^{2+} ions from the surrounding medium, was not affected by treatment with HMM-loaded liposomes (data not shown).

Ca^{2+} ions are known to control the stability of actin and myosin filaments and the mechanochemical reactivity of actomyosin (16,17). Control of the movement of Ca^{2+} ions by actomyosin would mean that the Ca^{2+}-actomyosin system is self-regulating.

This function of actomyosin, as inferred from the present data, does not exclude possible further roles of an actin filament network in regulating other cellular processes such as granule transport, or approach to the plasma membrane, prior to exocytosis.

ACKNOWLEDGEMENT : O.E. Harish thanks Mr. J. Friedman and Dr. P. Lelkes for their help in introducing her to the work with chromaffin cells and the liposome technique.

REFERENCES

1. Trifaro, J.M., Lee, R.W.H., Kenigsberg, R.L. and Côté, A. (1980) in Advances in the Biosciences, Vol. 36, Eds. F. Isumi, M. Oka, K. Kumakura. Pergamon Press, pp.151-158.
2. Pollard, H.B., Creutz, C.E., Fowler, V., Scott, J. and Pazoles, C.J. (1981) in Symposia on Quantitative Biology, Vol. XLVI, Cold Spring Harbor Laboratory, pp.819-834.
3. Lee, R.W.H. and Trifaro, J.M. (1981) Neuroscience 6, 2087-2108.
4. Friedman, J.E., Lelkes, P.I., Rosenheck, K. and Oplatka, A. (1980) Biochem.Biophys.Res.Comm. 96, 1717-1723.
5. Friedman, J.E., Lelkes, P.I., Rosenheck, K. and Oplatka, A. (1981) Abstracts of the International Conference on Molecular Neurobiology of Peripheral Catecholaminergic Systems held in IBIZA, 5-10th September 1981, p.158.
6. Baker, P.F. and Knight, D.E. (1978) Nature 276, 620-622.
7. Schneider, A.S., Herz, R. and Rosenheck, K. (1977) Proc.Natl.Acad.Sci. USA 74, 5036-5040.
8. Barenholtz, Y., Amselem, S. and Lictenberg, D. (1979) FEBS Lett. 99, 210-214.
9. Lelkes, P.I. (1983) in Liposome Technology, Vol. I, CRC Press, in press.
10. Gasko, D.D., Knowles, A.F., Shertzer, H.G., Suolinna, E.M. and Racker, E. (1976) Anal.Biochem. 72, 57-65.
11. Weeds, A.C. and Pope, B. (1977) J.Mol.Biol. 111, 129-157.
12. Oka, M., Isosaki, M. and Watanabe, J. (1981) Ref. 5, p.2936.
13. Kilpatrick, D.L., Slepetis, R.J., Corcoran, J.J. and Kirshner, N. (1982) J.Neurochem. 38, 427-435.
14. Reuter, H. (1983) Nature 301, 569-574.
15. Borejdo, J. and Oplatka, A. (1976) Biochim.Biophys.Acta 440, 241-246.
16. Borejdo, J., Muhlrad, A., Leibovich, S.J. and Oplatka, A. (1981) Biochim.Biophys.Acta 667, 118-131.
17. Kendrick-Jones, J., Tooth, P., Taylor, K.A. and Scholey, J.M. (1981) in Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLVI, pp.929-938.