Vol. 95, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 31, 1980 Pages 650-657

IONOPHORE-MEDIATED CALCIUM EXCHANGE DIFFUSION IN LIPOSOMES

M. Deleers and W.J. Malaisse

Laboratory of Experimental Medicine, Brussels University Medical School, Brussels, Belgium

Received June 10, 1980

SUMMARY: The exchange diffusion of ^{45}Ca in multilamellar liposomes containing the antibiotic ionophore A23187 is enhanced in a dose-related fashion at increasing concentrations of external Ca^{2+} or at increasing A23187/lipid molar ratios. An increase in fluidity of the lipid bilayer augments the permeability to Ca by facilitating both the formation and mobility of the Ca-ionophore complexes.

The transport of Ca by native or exogenous ionophores represents one modality for the passage of this cation across biological membranes. For instance, it was recently proposed that the process of Na-Ca counter transport, which accounts for the extrusion of Ca against its electrochemical gradient across the plasma membrane of cells (1,2), may be mediated by native ionophores (3-5). Likewise, certain ionophores may participate in a process of Ca-Ca exchange across biological membranes (6). In the present study, based on the use of liposomes as a model for biological membranes (7-8), we have characterized the influence of the Ca^{2+} gradient and the membrane fluidity upon the process of ionophore-mediated Ca exchange diffusion.

MATERIAL AND METHODS

Multilamellar liposomes were prepared from a mixture of dimyristoyl-phosphatidylcholine (DMPC) or distearoyl-phosphatidylcholine (DSPC) and cholesterol (molar ratio 7:2) solubilized in chloroform together with variable amounts of the antibiotic ionophore A23187. After complete evaporation of the solvent, 3 ml of a Tris-HCl buffer (20 mM; pH 7.4) containing NaCl (120 mM) and $^{45}CaCl_2$ (0.1 mM; 26 μCi/ml) was added to 20 mg of the dry film of lipids and vigorously shaken above transition temperature. After successive and rapid washes, the vesicles were resuspended in 1.0 ml of the same Tris-HCl buffer (except for the substitution of

0006-291X/80/140650-08$01.00/0

^{45}Ca by ^{40}Ca and change in $CaCl_2$ concentration) and incubated at room temperature (approximately 25°C). Samples of the latter buffer were removed at suitable intervals after rapid centrifugation of the liposomes, and examined for their radioactive content by liquid scintillation. Assuming that the initial decrease in ^{45}Ca content of the liposomes occured in an exponential manner, the initial permeability expressed as s^{-1} was taken as the $\ln(C_0/C_{10})/600$, formula in which C_0 an C_{10} represent the liposome content in ^{45}Ca at time zero and at the 10th min (600 s) of incubation, respectively. In the present system, the viscosity of the DSPC:cholesterol and DMPC:cholesterol liposomes as measured at 25°C with an Elscint MV1a microviscosimeter (9), averaged 5.06 ± 0.38 and 2.54 ± 0.29 P, respectively.

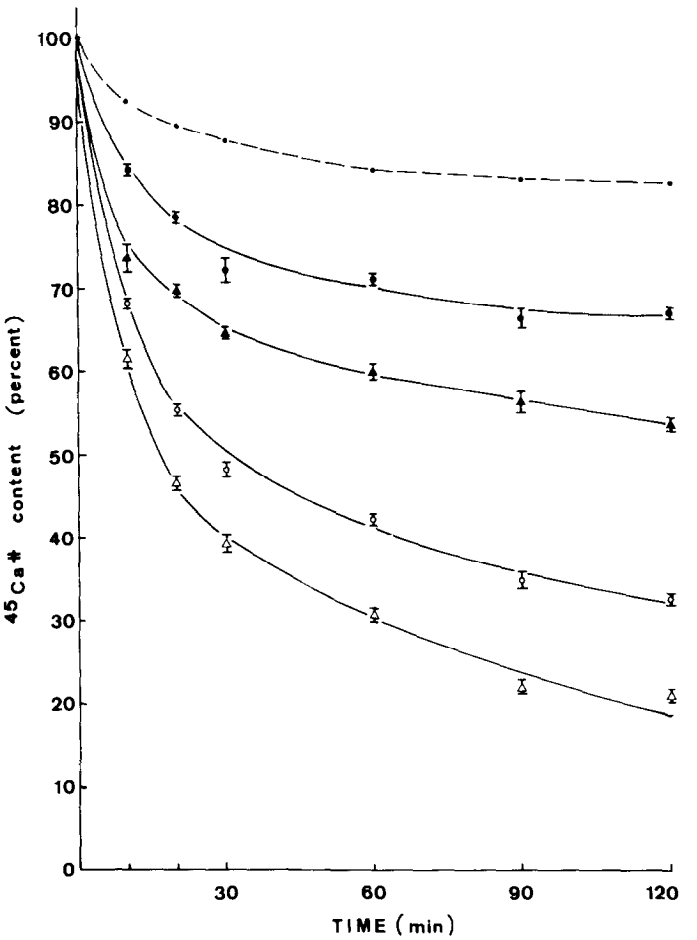

Fig. 1. Time course for the decrease in ^{45}Ca content of DMPC:cholesterol liposomes containing (solid lines) A23187 (0.31 mole of A23187/100 moles of lipid). The increase in the rate of ^{45}Ca efflux was obtained by raising the external Ca^{2+} concentration from 10^{-6} (closed circles) to 10^{-5} (closed triangles), 10^{-4} (open circles) and 10^{-3}M (open triangles). The dotted line refers to control experiments performed with liposomes devoid of A23187 and incubated in the absence of any Ca^{2+} gradient. Mean values (± SEM) refer to 4 individual experiments.

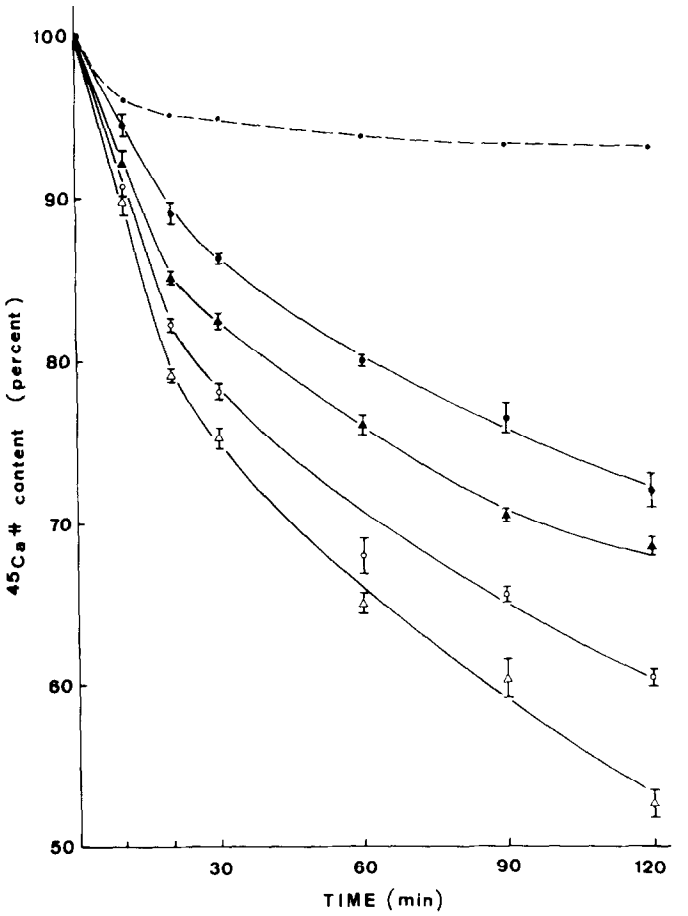

Fig 2. Time course for the decrease in ^{45}Ca content of DSPC:cholesterol liposomes containing (solid lines) A23187 (1.0 mole of A23187/100 moles of lipid). Same presentation as in Fig. 1.

RESULTS

When the liposomes contained no A23187 and in the absence of any Ca^{2+} gradient, little ^{45}Ca was released by the liposomes (Fig. 1). The presence of A23187 in the liposomes considerably increased the rate of ^{45}Ca release. As the concentration of external Ca^{2+} was raised from 10^{-6} to 10^{-3}M, a dose-related increase in A23187-mediated ^{45}Ca transport was observed.

The results illustrated in Fig. 1 were obtained with fluid liposomes composed of DMPC:cholesterol. In rigid liposomes composed of DSPC:cholesterol, the influence of A23187 and of the external

Ca^{2+} concentration upon ^{45}Ca release was qualitatively the same as in fluid liposomes (Fig. 2 and 3).

Several quantitative differences were observed between fluid and rigid liposomes. First, at given concentrations of A23187 and external Ca^{2+}, the rate of ^{45}Ca release was much higher in fluid than rigid liposomes. Thus, at a given external Ca^{2+} concentration,

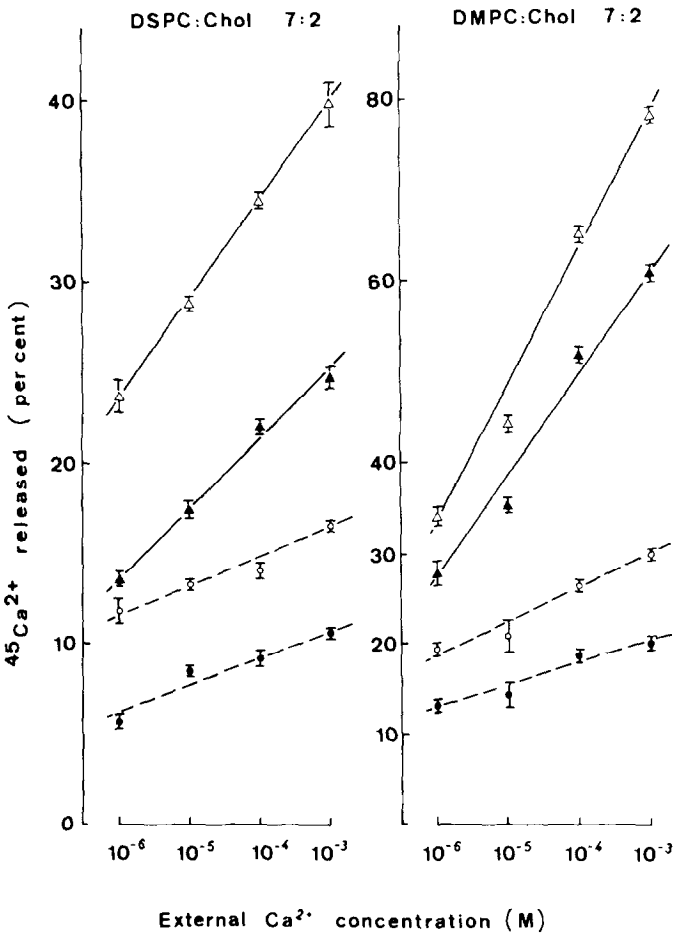

Fig. 3. Influence of the external Ca^{2+} concentration upon the amount of ^{45}Ca released by rigid or fluid liposomes. The A23187/lipid molar ratio amounted to either 0.31 % (dotted lines) or 1.00 % (solid lines) in the rigid liposomes (DSPC:cholesterol, left panel), and to either 0.10 % (dotted lines) or 0.31 % (solid lines) in the fluid liposomes (DMPC:cholesterol, right panel). The experimental data were collected after either 30 min (closed symbols) or 90 min (open symbols) incubation. Mean values (± SEM) are expressed in percent of the initial ^{45}Ca content of the liposomes, and refer to 4 individual observations. Note the difference in scale of the ordinates in the right and left panel, respectively.

Table 1. Mean values for the initial permeability in two classes of liposomes containing variable amounts of A23187(mol/mol of lipid) and incubated at increasing concentrations of external Ca^{2+}. In each class of liposomes and at each concentration of external Ca^{2+}, the change in Ca transport evoked by the same relative increase ($x \sqrt{10}$) in A23187 content was judged from the ratio (R) between the high and low permeability.

liposomes	DSPC:cholesterol (7:2)		DMPC:cholesterol (7:2)	
A23187	0.31 %	1.00 %	0.10 %	0.31 %
External Ca^{2+}	Initial permeability ($s^{-1}. 10^{-5}$)			
10^{-6} M	4.85	9.43(R=1.94)	10.68	28.17(R=2.64)
10^{-5} M	6.99	13.92(R=1.99)	14.15	50.97(R=3.60)
10^{-4} M	8.12	16.16(R=1.99)	18.60	63.96(R=3.44)
10^{-3} M	10.11	17.84(R=1.76)	19.74	80.99(R=4.10)

a ten-fold increase in A23187 concentration (from 0.1 to 1.0 mole of A23187/100 moles of lipid) was required to achieve the same permeability in rigid as in fluid liposomes (Table 1). Second, the same relative increase in A23187 concentration provoked a much greater increase of ^{45}Ca release in fluid than rigid liposomes, whatever the external Ca^{2+} concentration (Table 1). Last, in fluid liposomes, the relative increase in permeability attributable to a given increase in ionophore content was more marked at high than at low external Ca^{2+} concentration (Table 1). Such was not the case in rigid liposomes. The augmentation of ^{45}Ca transport observed at increasing concentrations of external Ca^{2+} was almost the same in fluid and rigid liposomes, provided that the A23187 content was selected in order to achieve a comparable permeability in these two types of liposomes.

DISCUSSION

The present findings emphasize the relevance of three distinct factors in the regulation of ionophore-mediated Ca transport across lipid bilayers, namely the ionophore content of the lipid

matrix, the external concentration of Ca^{2+}, and the fluidity of the artificial membrane.

The rate of ^{45}Ca transport was higher when either the ionophore content of the liposomes, the external Ca^{2+} concentration or the fluidity of the lipid phase was increased. Several observations illustrate the interaction between these three factors. The change in fluidity may affect the mobility of the Ca-ionophore complex across the bilayer. However, since the same relative increase in A23187 content provoked a greater increase in permeability in fluid than in rigid liposomes, an increase in fluidity may also facilitate the formation of the Ca-ionophore complex, presumably by increasing the lateral mobility of the ionophore. It is indeed well established that, in mediating Ca translocation into or across a hydrophobic domain, two molecules of A23187 are required to complex each atom of Ca (10, 11). The present hypothesis is also compatible with the mean distance between ionophoretic molecules which, in the present system, was estimated to range from 6.1 to 10.9 and 19.3 nm as the A23187 lipid molar ratio was decreased from 1.00 to 0.31 and 0.10 percent, respectively. Such distances correspond to the interposition between two molecules of ionophore of respectively 7, 13 and 22 lipid molecules in each leaflet of the bilayer.

Calcium itself apparently failed to interfere with the lateral mobility of the ionophore. Indeed the same increase in external Ca^{2+} concentration provoked the same increase in ^{45}Ca transport in fluid and rigid liposomes, provided that their A23187 content was chosen to yield a comparable efficiency in Ca transport.

In the fluid liposomes, the relative increase in permeability due to a given increase in ionophore content was itself dependent on the external Ca^{2+} concentration, being most marked at the highest Ca^{2+} level. This phenomenon is likely to be due the extension of

the Ca-Ca exchange diffusion process to the internal bilayers of the multilamellar liposomes. Such an extension may occur more rapidly in fluid liposomes rich in A23187 and exposed to high concentrations of external Ca^{2+}.

The rate of ^{45}Ca extrusion from the liposomes was strikingly increased when the external Ca^{2+} concentration was raised. Such a phenomenon, which was already observed in the case of X537A-mediated Ca transport in the Pressman cell (6), clearly indicates that the monodirectional transport of Ca is not merely driven by the Ca^{2+} gradient across the lipid bilayer. Indeed, in such a case, the rate of Ca^{2+} outflow from the liposomes would decrease as the external Ca^{2+} is increased. Since just the opposite picture was observed, our findings emphasize the view that the ionophore-mediated transport of Ca^{2+} against its chemical gradient is favoured by an increase in such a gradient. In other words, Ca may be better able to cross biological membranes by ionophoresis when moving into a medium of high as distinct from low Ca^{2+} concentration. In this respect, the present system may provide a tool for the study of Ca-stimulated Ca release from cellular organelles.

In physiological terms, our data also reveal that a change in membrane fluidity may represent an important factor in the environmental regulation of Ca transport across biological membranes.

ACKNOWLEDGEMENTS

This work was supported in part by grants of the Belgian Foundation for Scientific Medical Research and Belgian Institute for Scientific Research in Industry and Agriculture. The authors are grateful to Mrs. M. Mahy and J. De Ligne for technical assistance and secretarial help, respectively.

REFERENCES

1. Blaustein, M.P. (1974) Rev. Physiol. Biochem. Pharmacol. 70, 33-82.
2. Blaustein, M.P., and Russel, J.M. (1975) J. Memb. Biol. 22, 285-312.
3. Malaisse, W.J., and Couturier, E. (1978) Nature 275, 664-665.

4. Malaisse, W.J., Anjaneyulu, K., Anjaneyulu, R., and Couturier, E. (1980) Mol. Cell. Biochem. 30, 67-70.
5. Anjaneyulu, K., Anjaneyulu, R., and Malaisse, W.J. (1980) J. Inorg. Biochem., in press.
6. Couturier, E., and Malaisse, W.J. (1980) J. Inorg. Biochem. 12, 57-69.
7. Papahadjopoulos, D., and Kimelberg, H.K. (1973) in Progress in Surface Science (Pergamon Press) 4, 141-232.
8. Kimelberg, H.K., and Mayhew, E.G. (1978) C.R.C Critical Reviews in Toxicology 6, 25-79.
9. Shinitzky, M., and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367-394.
10. Pfeiffer, D.R., Reed, P.W., and Lardy, M.A. (1974) Biochemistry 13, 4007-4014.
11. Malaisse, W.J., Valverde, I., Devis, G., Somers, G., and Couturier, E. (1979) Biochimie 61, 1185-1192.