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Na^+-Ca^{2+} EXCHANGE ACTIVITY IN RABBIT LYMPHOCYTE PLASMA MEMBRANES

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Plasma membranes of rabbit thymus lymphocytes accumulated Ca^{2+} when a Na^+ gradient (intravesicular > extravesicular) was formed across the membranes. Dissipation of the Na^+ gradient by the addition of Na^+ to the external medium decreased Ca^{2+} uptake. Ca^{2+} preloaded into the lymphocytes was extruded when Na^+ was added to the external medium. The Ca^{2+} uptake decreased at acidic pH but increased at alkaline pH (above 8) and the activity was saturable for Ca^{2+} (apparent K_m for Ca^{2+} was 61 μM and apparent V_{max} was 11.5 nmol/mg protein per min). Na^+-dependent uptake of Ca^{2+} was inhibited by tetracaine and verapamil, and partially inhibited by La^{3+}. The uptake was not influenced by orthovanadate.

Introduction

It is well known that Ca^{2+} is involved in the responses of lymphocytes to several kinds of mitogen. One of the typical phenomena is that stimulation of Ca^{2+} influx accompany lectin stimulation of lymphocytes [1–6].

In recent years, two kinds of Ca^{2+}-pumping system have been demonstrated in cell membranes [7–11]: one is Ca^{2+}-pumping ATPase; the other is an Na^+-Ca^{2+} exchange system. In lymphocyte plasma membranes, Ca^{2+}-ATPase has been demonstrated [12–14], but the presence and characteristics of an Na^+-Ca^{2+} exchange system have not yet been determined.

I have studied the Na^+-Ca^{2+} exchange system in lymphocyte plasma membranes in order to understand better the Ca^{2+} activity and Ca^{2+}-extrusion mechanism in the lymphocytes.

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

Methods

Preparation of plasma membranes

Plasma membranes were prepared by a combination of the methods of Schmidt-Ullrich et al. [15] and Snary et al. [16] with slight modifications. Rabbit thymuses were collected immediately after death and suspended in buffer 1 (10 mM Tris-HCl (pH 7.4)/150 mM NaCl/1 mM $MgCl_2$/50 μg/ml streptomycin/50 units/ml penicillin G) at 0°C. The subsequent procedures were carried out at 0°C. Tissues were chopped into small pieces, homogenized in a Teflon-glass homogenizer with two strokes and passed through a stainless-steel mesh. The filtrate was then centrifuged at $80 \times g$ for 10 min. The resultant pellet was suspended in hypotonic buffer (17 mM Tris-HCl buffer (pH 7.2)/140 mM NH_4Cl/0.5 mM $MgCl_2$). After centrifugation at $80 \times g$ for 10 min, the pellet was washed three times with buffer 1. The washed cells were resuspended in 10 mM Hepes-KOH buffer (pH 7.4)/75 mM KCl/65 mM NaCl/0.25 mM $MgCl_2$ and homogenized in a Dounce homogenizer with a tight-fitting pestle. The homogenate was centri-

fuged at 4000 × *g* for 15 min and the supernatant was again centrifuged at 30000 × *g* for 40 min. The pellet (plasma membranes and microsomes) was suspended in 50 mM Tris-HCl buffer (pH 7.4), layered onto a 20–36% linear sucrose gradient and centrifuged at 110000 × *g* for 12 h. Membranes recovered from 21–25% sucrose fraction were used as plasma membrane fraction.

Analytical methods

Several marker enzymes for intracellular organelles and plasma membranes were assayed: 5′-Nucleotidase (EC 3.1.3.5) was determined with 0.5 mM levamisole [17]; alkaline phosphatase (EC 3.1.3.1) [18]; glucose-6-phosphatase (EC 3.1.3.9) [19]; NADH-diaphorase (EC 1.6.4.3) [20]; succinate dehydrogenase (EC 1.3.99.1) [21]; acid phosphatase (EC 3.1.3.2) [22], without addition of Triton X-100. Liberated inorganic phosphate was determined by the method of Ames [23].

Protein was estimated according to Lowry et al. [24] using bovine serum albumin as standard.

Assay of Ca^{2+}-transport

Na^+ was loaded inside the plasma membrane vesicles by incubation in Na^+-loading medium (100–200 mM NaCl/100 mM Tris-HCl buffer (pH 7.8)) and incubated at 37°C for 30 min. The Na^+-loaded vesicles were centrifuged at 110000 × *g* for 20 min. The resultant pellet was used for transport assay. 500 μl reaction mixture (100 mM Tris-HCl buffer (pH 7.8)/400 mM sucrose/40 μM $CaCl_2$ (with $3.7 \cdot 10^4$ Bq $^{45}CaCl_2$)) and membranes (protein, 50–100 μg/tube) were left to stand for 30 s at 0°C. Reaction was started by elevating the temperature from 0 to 37°C. At appropriate time intervals, 60-μl samples were removed and filtered on a cellulose nitrate filter (pore size, 0.2 μm). The membrane vesicles on the filter were washed three times with 2 ml 400 mM sucrose/2 mM $MgCl_2$ and dried. The radioactivity of the filters was determined by liquid scintillation counting and uptake activities are expressed as nmol Ca^{2+}/mg protein.

Chemicals

Tris was obtained from Merck (F.R.G.) and Hepes was from Sigma Chemical Co. (U.S.A.). $^{45}CaCl_2$ (35 Ci/g) was purchased from New England Nuclear (U.S.A.).

Results and Discussion

When crude plasma membranes were centrifuged on linear 20–36% sucrose density gradients, the membranes were separated into two major bands. These two bands were isolated and the activities of several marker enzymes were assayed with these fractions: alkaline phosphatase and 5′-nucleotidase (plasma membranes); glucose-6-phosphatase and NADH-diaphorase (endoplasmic reticulum); acid phosphatase (lysosomes) and succinate dehydrogenase (mitochondria). As shown in Table I, activities of alkaline phosphatase and 5′-nucleotidase were concentrated in the light fraction (21–25% sucrose). The enzymatic characteristics of plasma membranes and distribution of these enzymes were very similar to those of other plasma membrane fractions determined by other investigators [15,25–27]. Therefore I used these fractions for the present study. Enzyme activities found in the heavy fraction indicated that this fraction was a mixture of plasma membranes and endoplasmic reticulum.

Lichtman et al. [13] had reported the presence of Ca^{2+}-ATPase in the plasma membrane of lymphocytes: therefore, I tested ATP-dependent Ca^{2+} uptake activity in the membrane fraction obtained in the present study. When ATP was added to the reaction mixture containing Ca^{2+}, Ca^{2+} was accumulated into the membrane in a linear fashion for 90 s.

TABLE I

DISTRIBUTION OF ENZYME ACTIVITIES IN THE PLASMA MEMBRANE FRACTION OBTAINED BY SUCROSE DENSITY GRADIENT CENTRIFUGATION

Specific activities are expressed as nmol of products liberated/mg protein per min.

	Light fraction		Heavy fraction	
	I	II	I	II
5′-Nucleotidase	211	194	137	103
Alkaline phosphatase	299	271	214	166
Glucose-6-phosphatase	10.7	9.7	45.0	24.5
NADH-diaphorase	160	146	245	154
Acid phosphatase	6.8	4.9	25.5	7.1
Succinate dehydrogenase	< 10	< 10	< 10	< 10

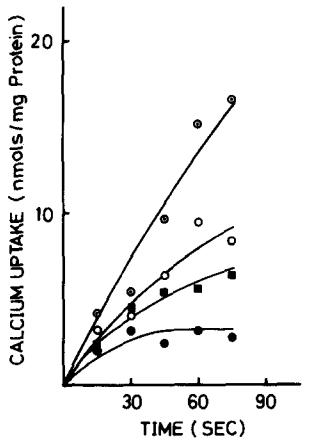

Fig. 1. Time-course of Ca^{2+} uptake by rabbit lymphocyte plasma membrane vesicles. Ca^{2+} uptake was assayed. The vesicles were loaded with 100 mM NaCl (○), 150 mM NaCl (⊙), 150 mM NaCl plus 1 μM monensin (■), and without NaCl (●). Each point represents the average of three experiments.

The Na^+-dependent Ca^{2+}-pumping activity of the membranes was then tested using Na^+-loaded vesicles. After washing the vesicles twice with 50 mM Tris-HCl buffer (pH 7.4), 100 and 150 mM Na^+ were loaded into the vesicles by passive diffusion. These Na^+-loaded vesicles were suspended in the reaction mixture containing $^{45}Ca^{2+}$ and incubated at 37°C. As shown in Fig. 1, Ca^{2+} accumulated in the vesicles within 90 s and the initial uptake rate and maximal amounts of uptake increased with increasing concentrations of intravesicular Na^+. When the Na^+ gradient was dissipated by 1 μM monensin, the uptake rate was markedly decreased. These results indicate that the outwardly directed Na^+ gradient drives Ca^{2+} uptake into these lymphocyte plasma membrane vesicles. When Na^+-loaded vesicles were suspended in the medium containing various amounts of Na^+ to reduce the magnitude of the Na^+ gradient, Ca^{2+} uptake decreased with increasing concentration of Na^+ in the extravesicular medium (Fig. 2).

The effect of external Na^+ on the rate of efflux of preloaded Ca^{2+} in the vesicles was then studied. After Na^+-loaded vesicles were allowed to accumulate Ca^{2+} for 30 s, Na^+ was added to the external medium, and efflux of Ca^{2+} from the vesicles was examined.

As shown in Fig. 3, preloaded Ca^{2+} was extruded from the vesicles by the inwardly directed Na^+ gradient. In this experiment, about 90% of the preloaded Ca^{2+} was extruded within 30 s.

A Lineweaver-Burk plot of Ca^{2+} uptake activity is shown in Fig. 4. The rate of Ca^{2+} uptake was a saturable function of the external Ca^{2+} concentra-

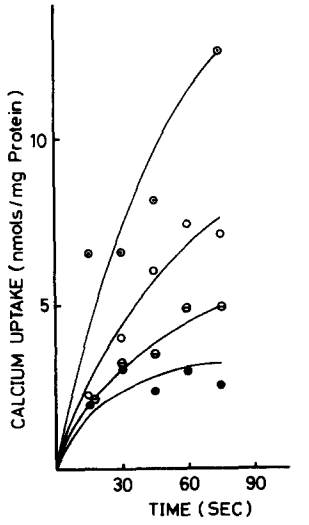

Fig. 2. Effect of external Na^+ on the Ca^{2+} uptake of Na^+-loaded vesicles. Plasma membrane vesicles were loaded with 200 mM NaCl and suspended in the media containing 200 mM NaCl (●), 100 mM NaCl (⊖), 50 mM NaCl (○), and without NaCl (⊙). Each point represent the average of two experiments.

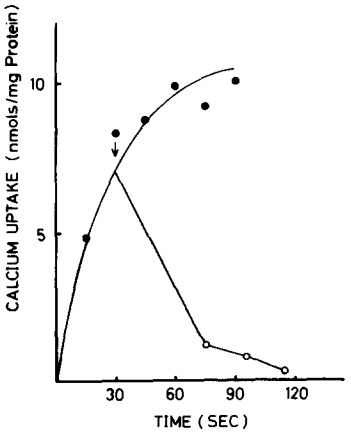

Fig. 3. Na^+-induced efflux of preloaded Ca^{2+} from the plasma membrane vesicles. Na^+-loaded (150 mM) vesicles were incubated in the medium containing $^{45}Ca^{2+}$. After 30 s of incubation, reaction mixture was devided into two parts and 200 mM NaCl (final concn.) was added (○) to one of them. Each point represents the average of two experiments.

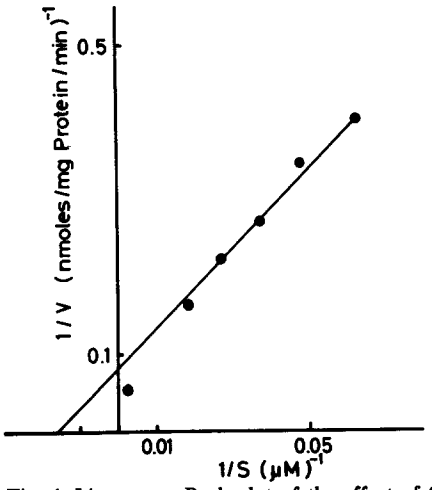

Fig. 4. Lineweaver-Burk plot of the effect of Ca^{2+} concentration on Na^{+}-Ca^{2+} exchange activity. Each point represents the average of two experiments.

tion present in the medium. The Lineweaver-Burk plot of the data showed an apparent K_m for Ca^{2+} at 61 μM and the apparent V_{max} at 11.5 nmol/mg protein per min.

The pH dependency of the Na^{+}-dependent uptake of Ca^{2+} was then studied. In order to avoid a transmembranous pH gradient, Na^{+} was loaded inside the vesicles at the same pH as in the uptake medium. Ca^{2+} uptake decreased at acidic pH, but increased at alkaline pH (above 8) (Fig. 5).

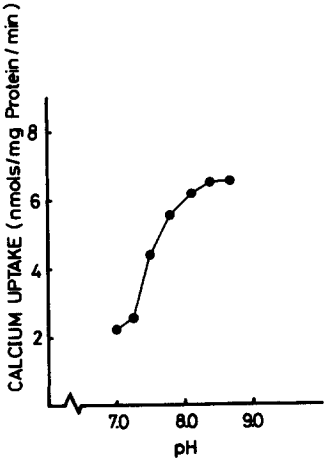

Fig. 5. pH dependency of Na^{+}-Ca^{2+} exchange activity. Each point represents the average of three experiments.

TABLE II

EFFECT OF CERTAIN DRUGS ON Na^{+}-Ca^{2+} EXCHANGE ACTIVITY OF LYMPHOCYTE PLASMA MEMBRANES

The values represent the average of three experiments.

	Concentration (mM)	Activity (%)
No addition	–	100
$LaCl_3$	0.5	85
Tetracaine HCl	2.5	40
Orthovanadate	1.0	100
Verapamil	5.5	40

The uptake was inhibited considerably by tetracain and verapamil, and only partially inhibited by La^{3+} (Table II). $LaCl_3$ is known to inhibit the Na^{+}-Ca^{2+} exchange in mitochondria and microsomes [28], but the Na^{+}-Ca^{2+} exchange reaction in the lymphocyte plasma membrane was not as sensitive to $LaCl_3$ as that in mitochondria or microsomes. Orthovanadate did not influence the uptake activity.

In the present study, I have shown that lymphocyte plasma membrane vesicles accumulate Ca^{2+} when an outwardly-directed Na^{+} gradient is imposed across the membranes. Extravesicular Na^{+} blocks Ca^{2+} uptake and stimulates the efflux rate of accumulated Ca^{2+} from the vesicles. Dissipation of the Na^{+} gradient by monensin (a cation exchange ionophore) leads to a decrease in the Ca^{2+} uptake rate. These results strongly support the existence of a Na^{+}-Ca^{2+} exchange system in lymphocyte plasma membranes.

The characteristics of the Na^{+}-Ca^{2+} exchange system were similar to those found in plasma membranes of other mammalian cells [28–32]. The activity of the Na^{+}-Ca^{2+} exchange system in the sarcolemmal vesicles [33] and squid axon [34] was found to be higher at alkaline pH than that at acidic pH. The K_m for Ca^{2+} in the exchange system in chick heart was 52 μM [32] and 38 μM in sarcolemmal vesicles of dog ventricles [33]. These values were not very different from that for lymphocyte plasma membranes presented in this study.

Vanadate is known to inhibit the activity of Ca^{2+}-ATPase in the presence of Mg^{2+} or K^{+}

[35–37]. I also studied the effect of vanadate on Na^+-Ca^{2+} exchange of lymphocytes. In this experiment, I set the concentration of Mg^{2+} at 1 mM to eliminate the inhibitory effect of Mg^{2+} on the exchange reaction. As presented in Table II, 1 mM vanadate did not inhibit the reaction of lymphocytes. On the other hand, when I tested the effect of La^{3+}, another inhibitor of Ca^{2+}-ATPase, on the Na^+-Ca^{2+} exchange of lymphocytes, the activity was found to be slightly decreased by the drug. La^{3+} might have some effect on the exchange reaction.

Recent evidence indicates that both Na^+-Ca^{2+} exchange and Ca^{2+}-ATPase exist in the plasma membranes of mammalian cells [7–11]. These two systems were also found to be associated with the plasma membrane fraction of lymphocyte, as described above. To elucidate the mechanism and regulation of the existing Ca^{2+}-transport activities, I feel that a more detailed study on the characteristics of the Na^+-Ca^{2+} exchange system should be required.

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References

1 Alford, R.H. (1970) J. Immunol. 104, 698–703
2 Allwood, G., Asherson, G.L., Davey, M.J. and Goodford, P.J. (1971) Immunology 21, 509–516
3 Whitney, R.B. and Sutherland, R.M. (1973) J. Cell. Physiol. 82, 9–20
4 Freedman, M.H., Martin, C.R. and Gomperts, B. (1975) Nature 255, 378–382
5 Jensen, P., Winger, L., Rasmussen, H. and Nowell, P. (1977) Biochim. Biophys. Acta 496, 374–383
6 Freedman, M.H. (1979) Cell. Immunol. 44, 290–313
7 Blaustein, M.P. (1977) Biophys. J. 20, 79–111
8 DiPolo, R. (1978) Nature 274, 390–392
9 Caroni, P. and Carafoli, E. (1980) Nature 283, 765–767
10 Schatzman, H.J. (1982) in Membrane Transport of Calcium (Carafoli, E. ed.), pp. 39–108, Academic Press, London
11 Gill, D.L., Grollman, E.F. and Kohn, L.D. (1981) J. Biol. Chem. 256, 184–192
12 Averdunk, R. and Günther, Th. (1980) Biochem. Biophys. Res. Commun. 97, 1146–1153
13 Lichtman, A.H., Segel, G.B. and Lichtman, M.A. (1981) J. Biol. Chem. 256, 6148–6154
14 Sarkadi, B., Enyedi, A., Szász, I. and Gárdos, G. (1982) Cell Calcium 3, 163–182
15 Schmidt-Ullrich, R., Ferber, E., Knufermann, H., Fischer, H. and Wallach, D.F.H. (1974) Biochim. Biophys. Acta 332, 175–191
16 Snary, D., Woods, F.R. and Crumpton, M.J. (1976) Anal. Biochem. 74, 457–465
17 Weaver, R.A. and Boyle, W. (1969) Biochim. Biophys. Acta 173, 377–388
18 Losa, G., Morell, A. and Barandun, S. (1982) Am. J. Pathol. 107, 191–200
19 Nordlie, R.C. and Aron, W.J. (1966) Methods Enzymol. 9, 619–625
20 Wallach, D.F.H. and Kamat, V.B. (1966) Methods Enzymol. 8, 164–172
21 King, T.E. (1963) J. Biol. Chem. 238, 4037–4051
22 Marique, D. and Hildebrand, J. (1973) Cancer Res. 33, 2761–2767
23 Ames, B.N. (1966) Methods Enzymol. 8, 115–118
24 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
25 Koizumi, K., Shimizu, S., Koizumi, T.K., Nishida, K., Sato, C., Ota, K. and Yamanaka, N. (1981) Biochim. Biophys. Acta 649, 393–403
26 Dumus, H. (1973) Biochim. Biophys. Acta 291, 93–106
27 Segel, G.B., Kovach, G. and Lichtman, M.A. (1979) J. Cell. Physiol. 100, 109–118
28 Schellenberg, G.D. and Swanson, P.D. (1981) Biochim. Biophys. Acta 648, 13–27
29 Reeves, J.P. and Sutko, J.L. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 590–594
30 Schellenberg, G.D. and Swanson, P.D. (1982) Biochim. Biophys. Acta 690, 133–144
31 Blaustein, M.P. and Nelson, M.T. (1982) in Membrane Transport of Calcium (Carafoli, E., ed), pp. 217–236, Academic Press, London
32 Wakabayashi, S. and Goshima, K. (1982) Biochim. Biophys. Acta 693, 125–133
33 Philipson, K.D., Bersohn, M.M. and Nishimoto, A.Y. (1982) Circulation Res. 50, 287–293
34 DiPolo, R. and Beaugé, L. (1982) Biochim. Biophys. Acta 688, 237–245
35 Bond, G.H. and Hudgins, P.M. (1980) Biochim. Biophys. Acta 600, 781–790
36 Barrabin, H. Garrahan, P.J. and Rega, A.F. (1980) Biochim. Biophys. Acta 600, 796–804
37 Rossi, J.P.F.C., Garrahan, P.J. and Rega, A.F. (1981) Biochim. Biophys. Acta 648, 145–150