Na+/Ca2+ exchange-mediated calcium entry in human lymphocytes.

M Balasubramanyam, … , J P Reeves, J P Gardner

J Clin Invest. 1994;94(5):2002-2008. https://doi.org/10.1172/JCI117553.

Research Article

Regulation of cytosolic Ca2+ and cytosolic Na+ is critical for lymphocyte cation homeostasis and function. To examine the influence of cytosolic Na+ on Ca2+ regulation in human peripheral blood lymphocytes, Ca2+ entry and cytosolic Ca2+ (measured with fura-2) were monitored in cells in which cytosolic Na+ was increased and/or the Na+ gradient was decreased by reduction of external Na+ concentration. Ouabain-treated cells (0.1 mM for 30 min at 37 degrees C), suspended in Na(+)-free medium, showed a 30-65% increase in Ca2+ uptake compared to cells in 140 mM Na+ medium. Enhanced Ca2+ influx was entirely dependent on ouabain pretreatment and reversal of the Na+ gradient. Na pump inhibition or Na ionophore addition and subsequent exposure to Na(+)-free medium resulted in a sustained elevation of cytosolic Ca2+. As preincubation of cells in Ca(2+)-free medium further enhanced the ouabain-dependent increase in cytosolic Ca2+, the effects of the microsomal Ca(2+)-ATPase inhibitor thapsigargin on Ca2+ influx and cytosolic Ca2+ were studied. Thapsigargin stimulated Ca2+ entry following ouabain pretreatment and reversal of the Na+ gradient; the effects of thapsigargin were retained in the presence of LaCl3, a potent inhibitor of store-dependent calcium influx pathways. These results show lymphocytes demonstrate Na+/Ca2+ exchange activity and suggest the Na+/Ca2+ exchanger modulates cytosolic Ca2+ following intracellular Ca2+ store depletion.

Na^+/Ca^{2+} Exchange-mediated Calcium Entry in Human Lymphocytes

M. Balasubramanyam,* Christine Rohowsky-Kochan,‡ John P. Reeves,§ and Jeffrey P. Gardner§

** Hypertension Research Center and Departments of ‡ Neurosciences, * Pediatrics, and § Physiology, University of Medicine and Dentistry–New Jersey Medical School, Newark, New Jersey 07103*

Abstract

Regulation of cytosolic Ca^{2+} and cytosolic Na^+ is critical for lymphocyte cation homeostasis and function. To examine the influence of cytosolic Na^+ on Ca^{2+} regulation in human peripheral blood lymphocytes, Ca^{2+} entry and cytosolic Ca^{2+} (measured with fura-2) were monitored in cells in which cytosolic Na^+ was increased and/or the Na^+ gradient was decreased by reduction of external Na^+ concentration. Ouabain-treated cells (0.1 mM for 30 min at 37°C), suspended in Na^+-free medium, showed a 30–65% increase in Ca^{2+} uptake compared to cells in 140 mM Na^+ medium. Enhanced Ca^{2+} influx was entirely dependent on ouabain pretreatment and reversal of the Na^+ gradient. Na pump inhibition or Na ionophore addition and subsequent exposure to Na^+-free medium resulted in a sustained elevation of cytosolic Ca^{2+}. As preincubation of cells in Ca^{2+}-free medium further enhanced the ouabain-dependent increase in cytosolic Ca^{2+}, the effects of the microsomal Ca^{2+}-ATPase inhibitor thapsigargin on Ca^{2+} influx and cytosolic Ca^{2+} were studied. Thapsigargin stimulated Ca^{2+} entry following ouabain pretreatment and reversal of the Na^+ gradient; the effects of thapsigargin were retained in the presence of $LaCl_3$, a potent inhibitor of store-dependent calcium influx pathways. These results show lymphocytes demonstrate Na^+/Ca^{2+} exchange activity and suggest the Na^+/Ca^{2+} exchanger modulates cytosolic Ca^{2+} following intracellular Ca^{2+} store depletion. (*J. Clin. Invest.* 1994. 94:2002–2008.)

Key words: human T lymphocytes • thapsigargin • Ca^{2+} uptake • store-dependent Ca^{2+} influx • intracellular Ca^{2+} stores

Introduction

The presence of the Na^+/Ca^{2+} exchanger has been suggested from experiments with rabbit lymphocyte vesicles (1) and human peripheral T lymphocytes and Jurkat T cells treated with Na^+/Ca^{2+} exchange inhibitors (2, 3). Indeed, modulation of cytosolic Ca^{2+} by Na^+/Ca^{2+} exchange mechanisms has been suggested as an important component of lymphocyte activation and/or proliferation responses (2). In contrast to these observations, Donnadieu and co-workers (4, 5) found no evidence of Na^+/Ca^{2+} exchange activity in T cell clones or the Jurkat T cell. To examine this issue further, we have employed a sensitive assay to detect Na^+/Ca^{2+} exchange activity in human lymphocytes, i.e., cytosolic Na^+-dependent Ca^{2+} influx.

Identifying Na^+/Ca^{2+} exchange activity in lymphocytes would increase our understanding of functional alterations in cation transport in several pathological conditions. White blood cells from subjects exhibiting essential hypertension (6–9) and diabetes mellitus (10, 11) have been studied to gain insight into cellular Na^+ and Ca^{2+} transport abnormalities. However, in order for lymphocytes to serve as a model for cellular Na^+ and Ca^{2+} homeostasis, they should express the cation transport mechanisms found in the target cells known to be affected in these disorders. Examples of target cells identified in hypertension and diabetes include the vascular smooth muscle and renal epithelial cells. These cells express plasma membrane Na^+-K^+-ATPase, Na^+-K^+-Cl^- cotransport, Na^+ H^+ antiport, Ca^{2+}-ATPase, and Na^+/Ca^{2+} exchange activities, and investigators have detected abnormal function or regulation of one or more of these transport systems in hypertension and diabetes (12–17). Inasmuch as lymphocytes have been shown to express all but Na^+/Ca^{2+} exchange activity, identifying this transport system would justify the use of this cell as a model for pathological alterations in Na^+ and Ca^{2+} transport.

Recently, we observed that Ca^{2+} efflux in human lymphocytes was stimulated by the presence of external Na^+ (18). Both the rate and threshold cytosolic [Ca^{2+}] for Ca^{2+} extrusion were increased in Na^+-containing medium, consistent with the presence of a Na^+/Ca^{2+} exchange mechanism in these cells. To obtain conclusive evidence for Na^+/Ca^{2+} exchange in lymphocytes, we have examined the effects of elevated cytosolic [Na^+] (produced by Na^+ pump inhibition or treatment with the Na^+ ionophore monensin) on Ca^{2+} influx.

Methods

Lymphocyte preparation. Unless noted, all reagents and drugs were from Sigma Chemical Co., St Louis, MO. Peripheral blood lymphocytes were obtained from healthy male volunteers by density-gradient centrifugation (Isoprep; Robbins Sci. Corp., Sunnyvale, CA). To limit monocyte contamination, carbonyl iron was used as described (18). Cells were suspended in Na^+-medium consisting of (in mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 20 Hepes, 10 glucose and 0.1% BSA (pH 7.4). Approval and informed consents were obtained from all subjects; the protocol was approved by the Institutional Review Board of UMDNJ-New Jersey Medical School. Phenotypic analysis of three lymphocyte preparations with dual fluorescence Ab labeling (19) on a FACScan® (Becton Dickinson Immunocytometry Systems, Mountain View, CA) indicated (mean±SE) 92±2% lymphocytes and 9±3% monocytes. $CD2^+$ T cells comprised 88±3% of all lymphocytes and B cells identified by the CD20 marker comprised 5±2% of all cells. Cell viability was > 95% as judged by trypan blue exclusion. Lymphocyte specific activators, including 3 μg/ml anti-CD3 (OKT3; Ortho Diagnostics, Raritan, NJ) and 50 μg/ml concanavalin A (type IV), increased cytosolic Ca^{2+} in preparations monitored with the Ca^{2+}-sensitive fluorescence dye fura-2.

Address correspondence to Dr. Jeffrey P. Gardner, Ph. D., Hypertension Research Center, MSB-F464, UMDNJ–New Jersey Medical School, 185 S. Orange Ave., Newark, NJ 07103.

Received for publication 22 March 1994 and in revised form 18 July 1994.

J. Clin. Invest.

0021-9738/94/11/2002/07 $2.00
Volume 94, November 1994, 2002–2008

Ca^{2+} influx measurements. Lymphocytes were preincubated with or without 0.1 mM ouabain in Na^+-medium for 30 min. To initiate Ca^{2+} uptake, aliquots (5 μl) of cells (1×10^8/ml) were diluted 20-fold into Na^+ medium or Na^+-free medium (*N*-methyl-D-glucamine adjusted to pH 7.4 with HCl [NMDG][1] or LiCl substituted isosmotically for NaCl) containing 10 μCi/ml $^{45}CaCl_2$ (final extracellular $[Na^+]$=6.7 mM in Na^+-free medium). Ouabain pretreatment was used to increase cytosolic Na^+ through inhibition of the Na^+-K^+-ATPase; in these and other ouabain pretreatment experiments, 0.1 mM ouabain was also included in the assay buffer. After 5-s–1-h incubation at 37°C, Ca^{2+} uptake was stopped by addition of 5 ml ice-cold Hepes medium (40 mM Hepes, pH 7.0, 100 mM $MgCl_2$, and 10 mM $LaCl_3$); extracellular radioactivity was removed by rapid filtration of cells on 0.45 μm filters (Millipore, Bedford, MA) with two additional washes of $LaCl_3$-containing Hepes-medium as described (20). Each data point represents the mean of *n* experiments derived from triplicate or quadruplicate measurements. For Ca^{2+} influx experiments, background counts (medium without cells) and 0 time Ca^{2+} uptake values were subtracted from all experimental time points (except Fig. 1, *inset,* where only background counts were subtracted).

Fluorescence measurements. Lymphocytes were incubated for 30 min at 37°C with 2 μM acetoxymethyl ester form of fura-2 (fura-2 AM) (Molecular Probes, Inc., Eugene, OR) in Na^+ medium or Na^+ medium minus $CaCl_2$ containing 0.3 mM EGTA (EGTA-Na^+ medium), with or without 0.1 mM ouabain as indicated. Cells were centrifuged for 5 s to remove external fura-2, resuspended in 50 μl of nominally Ca^{2+}-free Na^+ medium (medium with $CaCl_2$ omitted), and injected into cuvettes containing 3 ml nominally Ca^{2+}-free Na^+ medium or Ca^{2+}-free, Na^+-free medium (isosmotic LiCl substitution for NaCl; the final extracellular $[Na^+]$ was 2.3 mM, due to the 60-fold dilution of cells in Na^+-free medium). Fluorescence was monitored in stirred cells at 37°C in a Fluorolog II spectrofluorometer (SPEX Industries, Edison, NJ) after addition of $CaCl_2$ to bring extracellular Ca^{2+} to 1 mM. Excitation and emission wavelengths were 340–380 nm and 505 nm, respectively. Calibration of cytosolic $[Ca^{2+}]$ (21) was achieved by exposing lymphocytes to 100 μM digitonin in Na^+ medium (Rmax) followed by adding 15 mM EGTA (pH 8.0, Rmin). Fura-2 leakage at the completion of the experiment was assessed by adding 0.5 mM $MnCl_2$; as in previous studies, there was only a small (< 5%) decrease in the fluorescence spectra indicating minimal dye leakage (data not shown). Autofluorescence (determined in the presence of 0.5 mM $MnCl_2$ and 100 μM digitonin at the end of each experiment) was subtracted from fluorescence spectra prior to cytosolic $[Ca^{2+}]$ calculations.

Mn^{2+} can serve as a Ca^{2+} surrogate to study Ca^{2+} entry in fura-2–loaded cells (18, 22, 23). In these experiments, Mn^{2+} entering the cell binds to fura-2 and quenches its fluorescence; the quenching is monitored at the isosbestic wavelength (360 nm) of fura-2 such that changes in cytosolic Ca^{2+} do not affect the emission fluorescence. Mn^{2+} uptake was performed in fura-2–loaded cells exposed to Na^+ or Na^+-free medium containing 30 nM thapsigargin (LC Services, Woburn, MA), 0.5 mM $MnCl_2$ and 0.1–3 μM $LaCl_3$. Fluorescence was monitored at excitation and emission wavelengths of 360 and 505 nm, respectively, and results were normalized using 360 nm values obtained immediately before addition of $MnCl_2$.

Cytosolic Na^+ was measured in lymphocytes incubated with 5 μM sodium-binding benzofuran isophthalate acetoxymethyl ester (SBFI-AM) and 20% (wt/vol) Pluronic acid F-127 (Molecular Probes) in Na^+ medium for 60 min at 37°C, with or without 0.1 mM ouabain for the last 30 min. Fluorescence was measured at excitation and emission wavelengths 340–385 nm, and 505 nm, respectively, and calibration was performed by the gramicidin method (24). Autofluorescence was determined in unloaded cells and subtracted from fluorescence readings.

Statistical analysis. Repeated measures analysis of variance (ANOVA) was performed on an IBM compatible PC with a Statistical Analysis Systems (SAS) package, using the general linear model procedure (PROC GLM, Version 6.0; SAS Institute, Cary, NC). As Ca^{2+} uptake measurements were performed in triplicate or quadruplicate, a repeated measures (i.e., unbalanced) design using PROC GLM was utilized for this analysis (further details are given in reference 25). Students' *t* test was performed using Sigma Plot, version 4.0 (Jandel Scientific Software, San Rafael, CA). A P value < 0.05 was considered statistically significant. Data are reported as mean values±SE.

Results

Effect of reversing the Na^+ gradient on Ca^{2+} influx in lymphocytes. The Na^+/Ca^{2+} exchanger is a carrier system that mediates the transport of Ca^{2+} across the membrane in direct exchange for Na^+ (reviewed in reference 26). In most cells expressing Na^+/Ca^{2+} exchange activity, the physiological role of this system is to pump Ca^{2+} out of the cell ("forward" mode Na^+/Ca^{2+} exchange); however, under conditions where cytosolic Na^+ is increased, the exchanger can carry out the net influx of Ca^{2+} ("reverse mode" Na^+/Ca^{2+} exchange). To determine whether Na^+/Ca^{2+} exchange activity is present in lymphocytes, we employed $^{45}Ca^{2+}$ to study the nonsteady state accumulation of isotope by reverse mode Na^+/Ca^{2+} exchange. We first calculated Ca^{2+} uptake in cells pretreated with ouabain (to inhibit the Na^+ pump and raise cytosolic Na^+) and suspended in Na^+ or Na^+-free media. These conditions were imposed to decrease or reverse the inwardly directed Na^+ gradient that inhibits Ca^{2+} entry via the Na^+/Ca^{2+} exchanger. Lymphocytes exposed to 0.1 mM ouabain in Na^+ medium for 30 min demonstrated increased cytosolic $[Na^+]$ (42.9±2.4 vs 20.5±2.7 mM for untreated cells, $n = 5$). The time course of Ca^{2+} influx is shown in Fig. 1. Ca^{2+} influx was significantly increased (30–65%) in cells in Na^+-free medium vs. cells in Na^+ medium, consistent with the presence of a Na^+/Ca^{2+} exchange mechanism mediating Ca^{2+} entry. As shown in the inset to Fig. 1, Ca^{2+} influx increased linearly during the first 20–30 s in Na^+-containing and Na^+-free media. In general, the transient increase in Ca^{2+} uptake was followed by a lower, steady-state amount of Ca^{2+} influx. This decreased Ca^{2+} uptake was observed in cells in Na^+-free medium and to a lesser extent in cells in Na^+ medium. The difference may reflect loss of cytosolic Na^+ from the cells; ouabain-treated cells suspended in Na^+-free medium underwent a 35–40% decrease in cytosolic $[Na^+]$ during a 10-min period (data not shown). The data in Fig. 2 show that the enhanced uptake of Ca^{2+} in the Na^+-free medium was not observed if ouabain was omitted from the preincubation medium. Thus, both an increase in cytosolic $[Na^+]$ and reversal of the Na^+ gradient are necessary to demonstrate enhanced Ca^{2+} influx in lymphocytes.

Na^+/Ca^{2+} exchange-mediated increases in Ca^{2+} entry and cytosolic Ca^{2+} in Na^+-loaded lymphocytes. Fig. 3, *A* and *B* show the cytosolic Ca^{2+} profiles in cells that had been preincubated 30 min in Na^+ medium with (Fig. 3 *B*) or without (Fig. 3 *A*) 0.1 mM ouabain. Two traces are shown in each panel, corresponding to cells that were added to the cuvette containing either Na^+ medium (*solid line*) or Na^+-free medium (*dashed line*). Similar to the $^{45}Ca^{2+}$ findings, ouabain-treated cells showed an increase in cytosolic Ca^{2+} in the Na^+-free medium compared to 140 mM Na^+ medium, whereas no differences between cells in the two media were observed in the absence of ouabain treatment. Fig. 3, *C* and *D* show similar experiments

1. *Abbreviations used in this paper:* fura-2 AM, acetoxymethyl ester form of fura-2; NMDG, *N*-methyl-D-glucamine adjusted to pH 7.4 with HCl; SBFI-AM, sodium-binding benzofuran isophthalate acetoxymethyl ester.

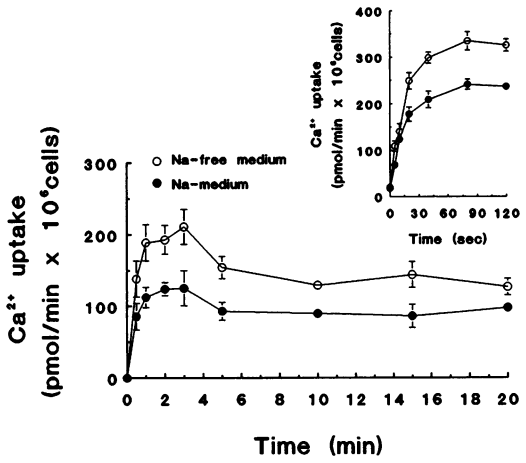

Figure 1. Effect of extracellular Na^+ on Ca^{2+} uptake by ouabain-treated lymphocytes. Lymphocytes were pretreated for 30 min at 37°C in Na^+ medium containing 0.1 mM ouabain and Ca^{2+} uptake assayed at indicated times in the presence of Na^+-containing (*closed circles*) or Na^+-free medium (*open circles,* NMDG substituted for NaCl). Ca^{2+} uptake at 0.5 and 1 h was unchanged from 20 min values (data not shown). Symbols represent means±SE for five experiments. Where error bars are not shown, the SE was smaller than the symbol. ANOVA (repeated measures, PROC GLM) indicated significant differences ($P = 0.0125$) in Ca^{2+} uptake values for cells in Na^+-containing vs Na^+-free medium. *Inset,* example of Ca^{2+} influx in the presence of Na^+ or absence of Na^+ from one subject. For this experiment, only the background counts were subtracted from data points. Linear regressions for the first four data points (i.e., 0, 5, 10, and 20 s) were: Na^+-medium; $y = 8.0(x) + 26.7$, $r = 0.98$, and for Na^+-free medium; $y = 10.9(x) + 33.7$, $r = 0.99$. Symbols represent means±SE for quadruplicate measurements.

using cells preincubated with or without ouabain in EGTA-Na^+-medium. The EGTA treatment decreases intracellular Ca^{2+} stores (Decreases in intracellular store size were estimated in fura-2-loaded cells resuspended in EGTA-Na^+ medium treated with 5 μM ionomycin and 100 nM thapsigargin. The peak of the cytosolic Ca^{2+} transient [reflecting the amount of Ca^{2+} in intracellular stores] was reduced 73.4±4.2% [plus ouabain] and 67.8±4.4% [minus ouabain] in cells incubated for 0.5 h in EGTA-Na^+ medium compared to cells incubated in Na^+ medium [n = 4 experiments].) and activates extracellular Ca^{2+} entry (see below), resulting in an initial time-dependent increase in cytosolic Ca^{2+} for all preincubation conditions. Ouabain-treated cells showed a larger and more sustained increase in cytosolic [Ca^{2+}] in Na^+-free medium than in Na^+ medium,

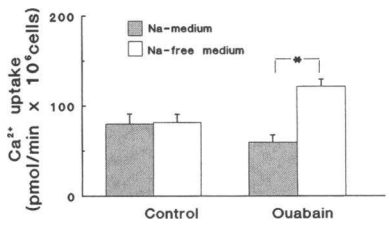

Figure 2. Effect of ouabain on Na^+-dependent Ca^{2+} uptake. Lymphocytes were preincubated for 30 min at 37°C in Na^+ medium with or without 0.1 mM ouabain and resuspended in Na^+- or Na^+-free medium (LiCl substituted for NaCl) containing $^{45}Ca^{2+}$. Ca^{2+} uptake was measured after 60 s; each experiment was performed in quadruplicate and averaged. Data represent mean values of five (control) and seven (ouabain) experiments. *$P = 0.0002$ (paired t test).

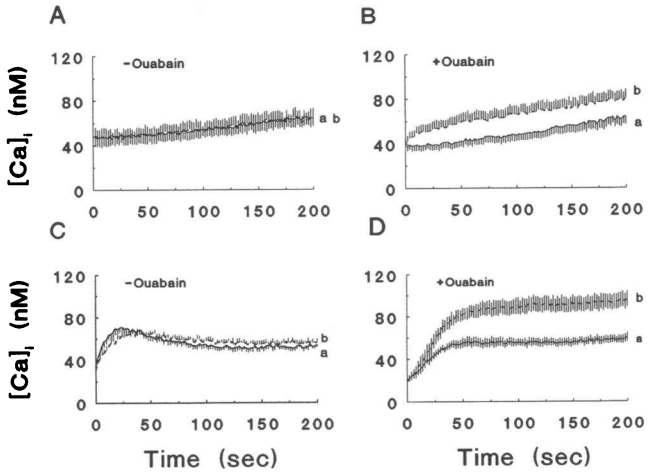

Figure 3. Effect of ouabain and extracellular Ca^{2+} depletion on the cytosolic Ca^{2+} profile in cells reexposed to Ca in Na^+-containing and Na^+-free medium. Lymphocytes were incubated for 30 min with fura-2 AM at 37°C in 1 mM Ca-containing media (*A* and *B*) or Ca-free medium (0.3 mM EGTA Na^+ medium (*C* and *D*), with or without ouabain as indicated. For experiments, cells were resuspended in nominally Ca^{2+}-free Na^+ medium and injected into cuvettes containing 3 ml of nominally Ca^{2+}-free a) Na^+ medium (*solid lines*), or b) or Na^+-free medium (LiCl substituted for NaCl; *broken lines*). Just before fluorescence monitoring, $CaCl_2$ was added (final extracellular [Ca^{2+}]= 1 mM) to the cuvette. Results are from five (*A* and *B*), eight (*C*), and ten (*D*) experiments; in these and subsequent figures, vertical bars denote SE.

whereas the changes in cytosolic [Ca^{2+}] in ouabain-free cells were independent of extracellular Na^+.

Na^+-dependent Ca^{2+} entry was also studied in ouabain-free cells using alternative procedures to raise cytosolic Na^+ (Fig. 4). In the first procedure, cytosolic [Na^+] was increased by incubating lymphocytes in K^+-free medium at 4°C to inhibit the Na^+ pump. Fig. 4 *A* shows the cytosolic Ca^{2+} profile of these cells suspended in Na^+-free medium (trace *c*), 40 mM (trace *b*) and 140 mM (trace *a*) Na^+ medium containing 1 mM Ca^{2+}. Following a rapid increase in Ca^{2+}, a sustained level of cytosolic [Ca^{2+}] is attained that is inversely related to the extracellular [Na^+]. The initial increase in cytosolic Ca^{2+} probably relates to Ca^{2+} entry through Na^+/Ca^{2+} exchange and store-dependent Ca^{2+} entry mechanisms, whereas the sustained phase reflects modulation of cytosolic Ca^{2+} by forward mode Na^+/Ca^{2+} exchange. For the second maneuver, Na^+-dependent Ca^{2+} entry in the presence of an active Na^+ pump was examined using monensin, a Na^+ ionophore (Fig. 4 *B*). Pretreatment of lymphocytes for 2 min with 10 μM monensin in Na^+ medium followed by exposure to 40-mM Na^+ medium (trace *b*) resulted in an approximate doubling in the cytosolic [Ca^{2+}] compared to ionophore-free cells (trace *a*). These data suggest Ca^{2+} influx is enhanced by increased cytosolic Na^+ and can be produced independent of ouabain treatment or Na^+ pump inhibition. The results support the conclusion of the Ca^{2+} flux studies and indicate that lymphocytes contain a Na^+/Ca^{2+} exchanger which modulates both Ca^{2+} entry and cytosolic Ca^{2+}.

Thapsigargin enhances Na^+-dependent Ca entry in ouabain-treated lymphocytes. Recently, specific inhibitors of the sarco(endo)plasmic reticulum Ca ATPase (SERCA), including thapsigargin (27), have been used to inhibit Ca^{2+} uptake by the endoplasmic reticulum, deplete intracellular stores of Ca^{2+} and

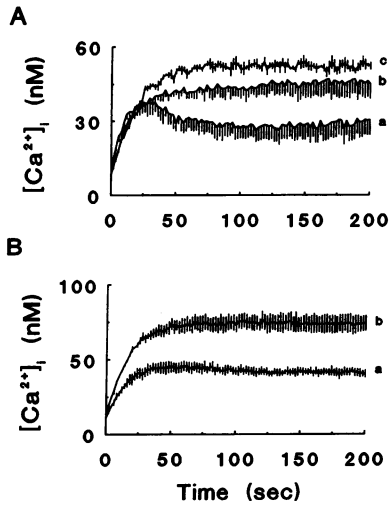

Figure 4. Demonstration of Na^+-dependent increases in cytosolic Ca^{2+} in the absence of ouabain. (*A*) Effect of Na^+ pump inhibition and extracellular Na^+ replacement on the cytosolic Ca^{2+} profile. Lymphocytes were loaded with fura-2 AM in nominally Ca^{2+}-free, K^+-free, Na^+ medium for 30 min (15 min at 37°, followed by 15 min at 4°C). Cells were resuspended in nominally Ca^{2+}-free Na^+ medium and subjected to (*a*) 140 nM Na^+ medium, (*b*) 40 mM Na^+ medium or (*c*) Na^+-free medium. $CaCl_2$ was added (final extracellular $[Ca^{2+}]$=1 mM) to cells at 0 s. Results are from three experiments. (B) Effect of monensin pretreatment and extracellular Na^+ on cytosolic Ca^{2+} profile. Fura-2 loaded lymphocytes were treated with vehicle (ethanol, 0.1% final volume) or 10 μM monensin in Na^+ medium for 2 min, resuspended in 50 μl of nominally Ca^{2+}-free, Na^+ medium and respectively subjected to (*a*) 40 mM Na^+-medium plus 0.1% ethanol, or (*b*) 40 mM Na^+ medium plus 10 μM monensin. $CaCl_2$ (final extracellular $[Ca^{2+}]$=1 mM) was added to cells at 0 s. Results are from four experiments. In one preparation in which cytosolic Na^+ was measured with SBFI, 10 μM monensin addition acutely (within 10 s) increased cytosolic Na^+ from 17 to 70 mM.

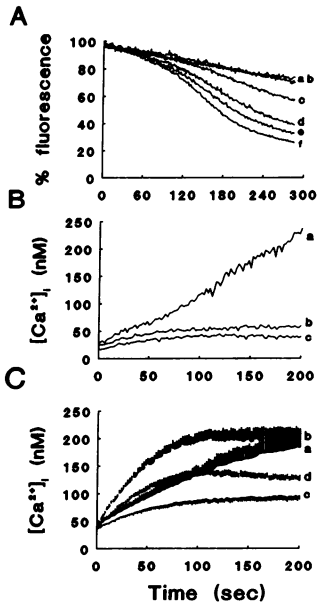

Figure 6. Thapsigargin enhances cytosolic Ca^{2+} independent of store-dependent Ca^{2+} entry. (*A*) Inhibition of thapsigargin-evoked Mn^{2+} uptake by $LaCl_3$. Lymphocytes were exposed to 30 nM thapsigargin and/or $LaCl_3$ in Na^+ medium and monitored at 360 nm as described in Methods. The decline in percent fluorescence is due to Mn^{2+} entry and quenching of cytosolic fura-2; an increase in the rate of decline results from enhanced Mn^{2+} uptake from the extracellular medium. Traces: (*a*) vehicle (0.1% DMSO), (*b*) thapsigargin plus 3 μM La^{3+}, (*c*) thapsigargin plus 1 μM La^{3+}, (*d*) thapsigargin plus 0.3 μM La^{3+}, (*e*) thapsigargin plus 0.1 μM La^{3+}, (*f*) thapsigargin alone. Initial 360 nm fluorescence values prior to addition of Mn^{2+} were $1.41\pm0.04 \times 10^6$ counts per s (n = 6). Results are representative of three experiments. (*B*) Inhibition of thapsigargin-evoked Ca^{2+} entry by $LaCl_3$. Lymphocytes were resuspended in nominally Ca^{2+}-free Na^+ medium and subjected to 30 nM thapsigargin and (*a*) 1 mM Ca^{2+}, (*b*) 1 mM Ca^{2+} plus 3 μM La^{3+}, or (*c*) 0.3 mM EGTA. Increases in cytosolic $[Ca^{2+}]$ in response to thapsigargin (measured as basal cytosolic $[Ca^{2+}]$ at 0 s subtracted from peak cytosolic $[Ca^{2+}]$ achieved at ~ 100 s) were 42.5 ± 7.4 nM and 35.5 ± 5.3 nM for cells exposed to $LaCl_3$ (trace *b*) and EGTA (trace *c*), respectively. Results are representative of six experiments. (*C*) Effect of ouabain pretreatment and extracellular Na^+ on thapsigargin induced cytosolic Ca^{2+} profile in the presence of $LaCl_3$. Lymphocytes were pretreated with ouabain in Na^+-medium, resuspended in nominally Ca^{2+}-free medium and injected into cuvettes containing ouabain, 30 nM thapsigargin and (*a*) Na^+ medium (*solid line*), (*b*) Na^+-free medium (*broken line*), (*c*) Na^+ medium plus 3 μM La^{3+} (*solid line*), or (*d*) Na^+-free medium plus 3 μM La^{3+} (*broken line*). Immediately before the experiment, $CaCl_2$ (final extracellular $[Ca^{2+}]$=1 mM) was added to the cuvette. Results are from six experiments.

activate extracellular Ca^{2+} entry (28). The intracellular signal–mediating Ca^{2+} entry is unknown (29, 30), but is identical to that produced in cells incubated in Ca^{2+}-free buffer (29). Ca^{2+} entry mediated by this process has been referred to as "capacitative Ca^{2+} entry" (31) or "store-dependent Ca^{2+} influx" (32). As thapsigargin addition results in a sustained increase in cytosolic Ca^{2+} in lymphocytes in Ca^{2+}-containing media (18, and see Fig. 6 *B*), we examined thapsigargin-induced Ca^{2+} entry in ouabain-treated or control cells suspended in Na^+ or Na^+-free medium. Cells were incubated with fura-2 AM in Ca^{2+}-containing medium, centrifuged, and resuspended in nominally Ca^{2+}-free buffer. In Fig. 5, traces *c* and *d* show that addition of 30 nM thapsigargin and Ca^{2+} (extracellular $[Ca^{2+}]$=1 mM) produced similar cytosolic Ca^{2+} responses in ouabain-free cells in both Na-containing and Na^+-free medium. The cytosolic Ca^{2+} profile for ouabain-treated cells suspended in Na^+ medium (trace *a*) was only slightly elevated compared to ouabain-free cells. Thapsigargin-induced increase in cytosolic Ca^{2+} was accelerated when ouabain-treated cells were assayed in Na^+-free medium (trace *b*). Thus, increased Ca^{2+} entry in thapsigargin-stimulated, ouabain-treated cells is sensitive to extracellular Na^+, suggesting that the Na^+/Ca^{2+} exchanger contributes to thapsigargin-induced Ca^{2+} entry under these conditions.

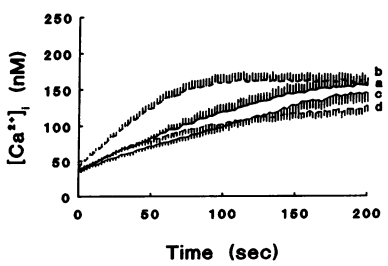

Figure 5. Effect of ouabain pretreatment and extracellular Na^+ on thapsigargin-induced cytosolic Ca^{2+} profiles. Lymphocytes were incubated during fura-2 loading period with or without ouabain in Na^+ medium, resuspended in 50 μl of nominally Ca^{2+}-free Na^+ medium and injected into cuvettes containing nominally Ca^{2+}-free (*a*) Na^+-medium plus ouabain (*solid line*), (*b*) Na^+-free medium plus ouabain (*broken line*), (*c*) Na^+ medium (*solid line*), or (*d*) Na^+-free medium (*broken line*). Prior to fluorescence monitoring, 30 nM thapsigargin and $CaCl_2$ (final extracellular $[Ca^{2+}]$=1 mM) were added to the cuvette. Results are from 10 experiments.

To determine the extent of Ca^{2+} uptake by Na^+/Ca^{2+} exchange in thapsigargin-treated cells, we utilized $LaCl_3$ to differentiate Ca^{2+} entry by store-dependent Ca^{2+} influx and Na^+/Ca^{2+} exchange pathways. Ca^{2+} entry by the store-dependent pathway is easily monitored by assaying Mn^{2+} uptake (18, 22, 23). In addition, in the presence of 1 mM Ca^{2+}, low concentrations of $LaCl_3$ (1–3 μM) inhibit store-dependent pathways without affecting Na^+/Ca^{2+} exchange activity (33). We thus used $LaCl_3$ to distinguish thapsigargin-stimulated Ca^{2+} (and Mn^{2+}) entry by store-dependent Ca^{2+} influx pathways and Ca^{2+} uptake via the Na^+/Ca^{2+} exchanger. In Fig. 6 *A*, thapsigargin-stimulated Mn^{2+} uptake and its inhibition by La^{3+} are shown. Mn^{2+} entry was sensitive to La^{3+} inhibition; at 3 μM La^{3+}, Mn^{2+} quenching

of fura-2 fluorescence was not different from control (thapsigargin-free) Mn^{2+} uptake. The profile of La^{3+} inhibition of Mn^{2+} uptake in lymphocytes suspended in Na^+-free medium was unchanged (data not shown). Moreover, La^{3+} inhibited extracellular Ca^{2+} entry in thapsigargin-stimulated, ouabain-free lymphocytes (Fig. 6 *B*). Thapsigargin-induced increases in cytosolic Ca^{2+} in cells in La^{3+}-containing medium (trace *b*) were similar to cells suspended in EGTA-Na^+ medium (trace *c*); both responses were significantly lower than the thapsigargin-induced increase in cytosolic Ca^{2+} in cells suspended in Ca^{2+}-containing, La^{3+}-free medium (trace *a*). Thus, 3 μM La^{3+} inhibits thapsigargin-stimulated Ca^{2+} uptake in the presence of extracellular Ca^{2+}. Finally, the effect of La^{3+} on thapsigargin-stimulated increases in cytosolic Ca^{2+} in ouabain-treated cells is shown in Fig. 6 *C*. La^{3+} reduced, but did not abolish, the increase in cytosolic Ca^{2+} in both Na-containing (see traces *a* and *c*) and Na^+-free medium (see traces *b* and *d*); inhibition by La^{3+} was more pronounced in Na medium than in Na^+-free medium. Thapsigargin increased the rate of Ca^{2+} entry and cytosolic Ca^{2+} by 100–150% in Na^+-free medium, even in the absence of Ca^{2+} entry via store-dependent Ca influx pathways (see traces *c* and *d*). The results indicate that in human lymphocytes, thapsigargin enhances Ca^{2+} entry through a La^{3+}-insensitive pathway that is stimulated by elevated cytosolic Na^+ and reduced extracellular [Na^+] (i.e., the Na^+/Ca^{2+} exchanger).

Discussion

This study examined Na^+-dependent Ca^{2+} entry in human peripheral blood lymphocytes. The main finding is that Na^+/Ca^{2+} exchange activity is present in lymphocytes, as both isotopic and fluorescence assays showed enhanced Ca^{2+} entry following maneuvers that increased cytosolic Na^+ and decreased extracellular [Na^+]. In addition, this work has developed the concept that Ca^{2+} entry and increased cytosolic Ca^{2+} are enhanced by the Na^+/Ca^{2+} exchanger following intracellular Ca^{2+} store depletion. These findings have important implications for Ca^{2+} homeostasis and cation transport in lymphocytes.

Wacholtz et al. (2, 3) have proposed that in T lymphocytes, the Na^+/Ca^{2+} exchanger mediates increased Ca^{2+} entry, resulting in interleukin 2 (IL-2) synthesis and IL-2 receptor expression following cell activation. Their findings were based on the effects of inhibitors of Na^+/Ca^{2+} exchange such as bepridil and amiloride analogs. These inhibitors, however, are not specific antagonists of Na^+/Ca^{2+} exchange and can have multiple effects, particularly when exposed to cells over a prolonged period, as in the experiments of Wacholtz et al. (2, 3). The findings of these investigators were further challenged by Donnadieu and co-workers (4, 5), who were unable to detect Na^+/Ca^{2+} exchange activity in T cell clones and Jurkat T cells. We observed increased Ca^{2+} uptake and cytosolic Ca^{2+} only after cytosolic [Na^+] was approximately doubled (by ouabain pretreatment) and cells were exposed to Na^+-free medium. These experimental conditions may explain the inability of some investigators to demonstrate Na^+/Ca^{2+} exchange-mediated Ca^{2+} entry in T lymphocytes or in Jurkat T cells (4, 5). In this regard, we were also unable to detect exchange activity in Jurkat T cells (34) using experimental protocols similar to those described in this report. These results suggest that expression and/or activity of the Na^+/Ca^{2+} exchanger differ between resting and transformed lymphocytes.

Potentiation of Ca^{2+} influx in lymphocytes by Na^+/Ca^{2+} exchange mechanisms following ouabain-pretreatment is similar to cardiac, skin, lung (35), and aortic tissues (36), and renal epithelial cells (37) and Chinese hamster ovary cells stably expressing the cardiac Na^+/Ca^{2+} exchanger (38). Assuming initial exchange-dependent Ca^{2+} uptakes of 106 pmol/[min $\times$ 10^6 cells] (extrapolated from 30-s values, Fig. 1) and a protein concentration of 30 $\mu g/10^6$ cells, Na^+/Ca^{2+} exchange activity in this peripheral lymphocyte preparation is $\sim$ 3.5 nmol/[min $\times$ mg]. Although this value probably underestimates actual transport activity, it is similar to skin and lung (1.4 and 2.6 nmol/[min $\times$ mg], respectively, 35), but 3–30-fold lower than cardiac (100 nmol/[min $\times$ mg], 35), aortic (22 nmol/[min $\times$ mg], 36), epithelial cells (11.3 nmol/[min $\times$ mg], 37) and transfected Chinese hamster ovary cells (70 nmol/[min $\times$ mg], 38). The decreased activity of this system may reflect low amounts of exchange protein (Preliminary attempts to identify the lymphocyte Na^+/Ca^{2+} exchanger by Western analysis were unsuccessful [M. Condrescu and J. Reeves, unpublished observations]. Although there may be several explanations for this result, we note that attempts to demonstrate the cardiac Na^+/Ca^{2+} exchanger in transfected CHO cells (38) were also unfruitful unless immunoprecipitation with ^{35}S-labeled proteins was performed.) or altered kinetic or regulatory properties of the lymphocyte Na^+/Ca^{2+} exchanger.

Lymphocytes demonstrated a transient increase in Ca^{2+} uptake that was more pronounced in Na^+-free than Na^+-containing medium. The most likely explanation for the transience of this effect is the time-dependent loss of cytosolic Na^+ in Na-free medium, reducing the outwardly directed Na^+ gradient and inhibiting Ca^{2+} influx. In addition, other factors influence regulatory and kinetic aspects of the Na^+/Ca^{2+} exchanger. For example, NaCl replacement with NMDG (see Fig. 1) results in Na^+/H^+ antiport inhibition and cell acidification, which could reduce Na^+/Ca^{2+} exchange mediated Ca^{2+} uptake (39, 40). However, when Ca^{2+} influx was measured in Li^+-substituted solutions to avoid cell acidification (Li^+ is countertransported in exchange for intracellular H^+), Ca^{2+} uptake in this solution was not enhanced, indicating acute Na^+/H^+ antiport inhibition has minimal effects on Ca^{2+} influx via the Na^+/Ca^{2+} exchanger. An additional factor is the presence of $MgCl_2$, which could decrease Ca^{2+} entry, as Mg^{2+} is a competitive inhibitor of Na^+/Ca^{2+} exchange (41, 42). Finally, in addition to forward mode Na^+/Ca^{2+} exchange, the plasma membrane Ca^{2+} pump participates in the extrusion of cytosolic Ca^{2+} (18, 43). Accelerated Ca^{2+} pump activity at higher Ca^{2+} levels would tend to diminish cellular Ca^{2+} accumulation.

Enhanced Ca^{2+} uptake was observed in ouabain-treated cells in both $NMDG^+$- and Li^+-substituted media. These results suggest Na^+/Ca^{2+} exchange–mediated Ca^{2+} influx in lymphocytes is independent of the substituted, extracellular cation. These experiments do not address effects of extracellular cation substitution on kinetic parameters of the Na^+/Ca^{2+} exchange, which could affect estimations of V_{max}.

Cytosolic Ca^{2+} experiments in fura-2–loaded cells supported the results of Ca^{2+} uptake studies, and shared the requirement for ouabain pretreatment and decreased extracellular Na^+ to distinguish Na^+/Ca^{2+} exchange-dependent Ca^{2+} entry. In some cells, low extracellular [Na^+] results in inositol trisphosphate generation, Ca^{2+} mobilization from intracellular stores and increases in cytosolic Ca^{2+} (44). A similar mechanism could explain the increase in cytosolic Ca^{2+} in ouabain-treated cells suspended in Na^+-free medium (Fig. 3 *B*). However, ouabain-

free cells in Na^+-free medium did not respond with a cytosolic Ca^{2+} transient. Furthermore, lymphocytes preincubated in ouabain and EGTA-Na^+ medium demonstrated enhanced cytosolic Ca^{2+} responses in Na^+-free medium. As this treatment decreases intracellular Ca^{2+} stores, increases in cytosolic Ca^{2+} in Na^+-free medium result from extracellular Ca^{2+} influx. This finding was further explored by treating lymphocytes with thapsigargin to inhibit SERCA-Ca^{2+} ATPase activity and activate store-dependent Ca^{2+} influx mechanisms. Ouabain pretreatment enhanced the rate and magnitude of Ca^{2+} entry in thapsigargin-treated lymphocytes. One possible explanation for this result is that the elevated cytosolic Na^+ inhibits Ca^{2+} efflux mediated by the exchanger, so that higher cytosolic [Ca^{2+}] are attained during Ca^{2+} entry via the store-dependent Ca^{2+} influx pathway in ouabain-treated cells. This interpretation emphasizes the importance of Na^+/Ca^{2+} exchange as a Ca^{2+} efflux mechanism in lymphocytes. Another explanation is that intracellular store depletion enhances Ca^{2+} entry not only through store-dependent Ca^{2+} channels (45), but also through the Na^+/Ca^{2+} exchanger. Even when La^{3+} was present to block the Ca^{2+} influx pathway (see Fig. 6 *C*), ouabain pretreatment and suspension of cells in Na^+-free medium enhanced the cytosolic Ca^{2+} profile. A similar observation was made in a recent study examining the effects of thapsigargin on Ca^{2+} uptake in transfected Chinese hamster ovary cells expressing the cardiac Na^+/Ca^{2+} exchanger (33).

The primary role of the Na^+/Ca^{2+} exchanger in lymphocytes is most likely cytosolic Ca^{2+} extrusion (18), for example, during Ca^{2+}-spiking events (45–47) or periods of elevated cytosolic [Ca^{2+}] following cellular activation. However, as discussed above, a role for Na^+/Ca^{2+} exchange-mediated Ca^{2+} entry during lymphocyte activation should also be considered. Dolmetsch and Lewis (45) recently reported low concentrations of thapsigargin (e.g., 10–40 nM) induced cytosolic Ca^{2+} oscillations in T lymphocytes that were dependent on partial emptying of intracellular Ca^{2+} stores and extracellular Ca^{2+} influx. Treatment of lymphocytes with thapsigargin and phorbol myristate acetate in vitro increased IL-2 production (a Ca^{2+}-dependent phenomenon) and expression of IL-2 receptors (48), indicating thapsigargin can stimulate cell proliferation. Breittmayer and colleagues (49) reported that microsomal Ca^{2+}-ATPase inhibition was closely associated with intracellular store emptying, inhibition of phosphatidylinositol synthesis and increased [^{3}H]thymidine incorporation in human peripheral T cells. As T cell antigens and lymphocytic mitogens mobilize Ca^{2+} from intracellular stores and activate Ca^{2+} influx pathways (50, 51), the Na^+/Ca^{2+} exchanger may play a role in generating a cytosolic Ca^{2+} signal sufficient for inducing cell proliferation.

Finally, lymphocytes are used by many investigators to study physiological (e.g., ion transport and volume regulation) and pathophysiological (6–11, 52, 53) phenomena. Oshima et al. (8) suggested a potential role for Na^+/Ca^{2+} exchange mediating increased cytosolic [Ca^{2+}] in lymphocytes isolated from essential hypertensive subjects. In light of recent findings suggesting that the Na^+-K^+-ATPase may be inhibited by endogenous ouabain (or a factor exhibiting ouabain-like activity) in some hypertensive subjects (16), increased cytosolic [Na^+] may indeed have a causal role in the regulation of cytosolic Ca^{2+} and/or Ca^{2+} content of intracellular stores. The evidence presented here affirms the exchanger is present in human lymphocytes, and suggests that this cell preparation may be useful for studying Na^+/Ca^{2+} exchange-related alterations in cytosolic Ca^{2+} and Na^+ in essential hypertension and other diseases.

Acknowledgments

We thank Donna Eiman for assisting with the phenotypic analysis and Dr. Abraham Aviv for helpful comments on the manuscript.

This work was supported by the National Institutes of Health (HL44196 and HL49932). M. Balasubramanyam is a Postdoctoral fellow of the American Heart Association, New Jersey Affiliate.

References

1. Ueda, T. 1983. Na^+/Ca^{2+} exchange activity in rabbit lymphocyte plasma membranes. *Biochim. Biophys. Acta.* 734:342–346.

2. Wacholtz, M. C., E. J. Cragoe, Jr., and P. E. Lipsky. 1992. A Na^+-dependent Ca^{2+} exchanger generates the sustained increase in intracellular Ca^{2+} required for T cell activation. *J. Immunol.* 149:1912–1920.

3. Wacholtz, M. C., E. J. Cragoe, Jr., and P. E. Lipsky. 1993. Delineation of the role of a Na^+/Ca^{2+} exchanger in regulating intracellular Ca^{2+} in T cells. *Cell. Immunol.* 147:95–109.

4. Donnadieu, E., G. Bismuth, and A. Trautmann. 1992. Calcium fluxes in T lymphocytes. *J. Biol. Chem.* 267:25864–25872.

5. Donnadieu, E., and A. Trautmann. 1993. Is there a Na^+/Ca^{2+} exchanger in macrophages and in lymphocytes? *Pflueg. Archiv. Eur. J. Physiol.* 424:448–455.

6. Edmondson, R. P. S., R. D. Thomas, P. J. Hilton, J. Patrick, and N. F. Jones. 1975. Abnormal leukocyte composition and sodium transport in essential hypertension. *Lancet.* i:1003–1005.

7. Ambrosioni, E., F. V. Costa, L. Montebugnoli, F. Tartagni, and B. Magnani. 1981. Increased intralymphocytic sodium content in essential hypertension: an index of impaired Na cellular metabolism. *Clin. Sci. (Lond.).* 61:181–186.

8. Oshima, T., H. Matsuura, K. Kido, K. Matsumoto, H. Fujii, S. Masaoka, M. Okamoto, Y. Tsuchioka, G. Kajiyama, and T. Tsubokura. 1988. Intralymphocytic sodium and free calcium and plasma renin in essential hypertension. *Hypertension (Dallas).* 12:26–31.

9. Ng, L. L., D. A. Fennel, and C. Dudley. 1990. Kinetics of the human leucocyte Na^+-H^+ antiport in essential hypertension. *J. Hypertens.* 8:533–537.

10. Alexiewicz, J. M., Z. Gaciong, M. Parise, F. Karubian, S. G. Massry, and V. M. Campese. 1992. Effect of dietary sodium intake on intracellular calcium in lymphocytes of salt-sensitive hypertensive patients. *Am. J. Hypertens.* 5:536–541.

11. Ng, L. L., D. Simmons, V. Frighi, M. C. Garrido, J. Bomford, and T. D. Hockaday. 1990. Leukocyte Na^+/H^+ antiport activity in type 1 (insulin-dependent) diabetic patients with nephropathy. *Diabetologia.* 33:371–377.

12. Lechene, C., M. Crabos, G. Bianchi, and H. Cantielo. 1989. Role physiologique de la pompe a sodium. Implications pour l'etude de l'hypertension arterielle. *Nephrologie.* 10:59–64.

13. Garay, R. P. 1987. Kinetic aspects of red blood cells sodium transport systems in essential hypertension. *Hypertension (Dallas).* 19[Suppl. I]:I-11–I-14.

14. Aviv, A. 1994. The cytosolic Ca^{2+}, Na^+/H^+ antiport, protein kinase C trio in essential hypertension. *Am. J. Hypertens.* 7:205–212.

15. Rosskopf, D., R. Dusing, and W. Siffert. 1993. Membrane sodium–proton exchange and primary hypertension. *Hypertension (Dallas).* 21:607–617.

16. Blaustein, M. P., and J. M. Hamlyn. 1991. Pathogenesis of essential hypertension. A link between dietary salt and high blood pressure. *Hypertension (Dallas).* 18[Suppl. III]:III-184–III-195.

17. Huot, S. J., and P. S. Aronson. 1991. Na^+/H^+ exchanger and its role in essential hypertension and diabetes mellitus. *Diabetes Care.* 14:521–535.

18. Balasubramanyam, M., M. Kimura, A. Aviv, and J. P. Gardner. 1993. Kinetics of Ca^{2+} transport across the lymphocyte plasma membrane. *Am. J. Physiol.* 265 (*Cell Physiol. 34*):C321–C327.

19. Rohowsky-Kochan, C., D. Eiman, T. Denny, J. Oleske, and S. D. Cook. 1994. Induction of autologous mixed lymphocyte culture responses by myelin basic protein-reactive T cell clones. *J. Neuroimmunol.* 50:59–70.

20. Reeves, J. P. 1988. Measurement of sodium-calcium exchange activity in plasma membrane vesicles. *Methods Enzymol.* 157:505–510.

21. Grynkiewicz, B., M. Poenie, and R. Y. Tsien. 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440–3450.

22. Hallam, T. J., and T. J. Rink. 1985. Agonists stimulate divalent cation channels in the plasma membrane of human platelets. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 186:175–179.

23. Minta, A., and R. Y. Tsien. 1989. Fluorescent indicators for cytosolic sodium. *J. Biol. Chem.* 264:19449–19457.

24. Gouy, H. D., S. B. Cefai, B. Christensen, P. Debre, and G. Bismuth. 1990. Ca influx in human T lymphocytes is induced independently of inositol phosphate production by mobilization of intracellular Ca^{2+} stores. A study with the Ca endoplasmic reticulum-ATPase inhibitor thapsigargin. *Eur. J. Immunol.* 20:2269–2275.

25. Cody, R. P., and J. K. Smith. 1991. Applied Statistics and the SAS Programming Language. Prentice Hall, Englewood Cliffs, NJ 403 pp.

26. Reeves, J. P. 1990. Sodium calcium exchange. *In* Intracellular Calcium Regulation. F. Bronner, editor. Wiley-Liss, Inc., New York 305–347.

27. Lytton, J., M. Westlin, and M. R. Hanley. 1991. Thapsigargin inhibits the sarcoplasmic or endoplasmic reticulum Ca^{2+}-ATPase family of Ca^{2+} pumps. *J. Biol. Chem.* 266:17067–17071.

28. Thastrup, O., P. J. Cullen, B. K. Drobak, M. R. Hanley, and A. P. Dawson. 1990. Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. *Proc. Natl. Acad. Sci. USA.* 87:2466–2470.

29. Randriamampita, C., and R. Y. Tsien. 1993. Emptying of intracellular Ca^{2+} stores releases a novel small messenger that stimulates Ca^{2+} influx. *Nature (Lond.).* 364:809–814.

30. Parekh, A. B., H. Terlau, and W. Stuhmer. 1993. Depletion of $InsP_3$ stores activates a Ca^{2+} and K^+ current by means of a phosphatase and a diffusible messenger. *Nature (Lond.).* 364:814–818.

31. Putney, J. W., Jr. 1991. The capacitative model for receptor-activated calcium entry. *Adv. Pharmacol.* 22:251–269.

32. Clementi, E., H. Scheer, D. Zacchetti, C. Fasolato, T. Possan, and J. Meldolesi. 1992. Receptor-activated Ca^{2+} influx. *J. Biol. Chem.* 267:2164–2172.

33. Reeves, J. P., J. P. Gardner, G. Chernaya, J. F. Aceto, and M. Condrescu. 1994. Thapsigargin inhibits calcium recycling in CHO cells transfected with the bovine sodium-calcium exchanger. *Biophys. J.* 66:148*A*. (Abstr.).

34. Gardner, J. P., and M. Balasubramanyam. 1994. Increased cytosolic free calcium (Ca_i), Ca mobilization and Ca turnover rate in Jurkat T lymphocytes. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 8(5-Part 2):*A*781. (Abstr.)

35. Wakabayashi, S., and K. Goshima. 1981. Kinetic studies on sodium-dependent calcium uptake by myocardial cells and neuroblastoma cells in culture. *Biochim. Biophys. Acta.* 642:158–172.

36. Lyu, R.-M., L. Smith, and J. B. Smith. 1992. Ca^{2+} influx via Na^+-Ca^{2+} exchange in immortalized aortic myocytes. I. Dependence on $[Na^+]_i$ and inhibition by external Na. *Am. J. Physiol.* 263(*Cell Physiol. 32*):C628–C634.

37. Lyu, R.-M., L. Smith, and J. B. Smith. 1991. Sodium-calcium exchange in renal epithelial cells: dependence on cell sodium and competitive inhibition by magnesium. *J. Membr. Biol.* 124:73–83.

38. Pijuan, V., Y. Zhuang, L. Smith, C. Kroupis, M. Condrescu, J. F. Aceto, J. P. Reeves, and J. B. Smith. 1993. Stable expression of the cardiac sodium-calcium exchanger in CHO cells. *Am. J. Physiol.* 264(*Cell Physiol. 33*):C1066–C1074.

39. DiPolo, R., and L. Beauge. 1982. The effect of pH on Ca^{2+} extrusion mechanisms in dialyzed squid axons. *Biochim. Biophys. Acta.* 688:227–245.

40. Philipson, K. D., J. S. Frank, and A. Y. Nishimoto. 1982. Effects of pH on Na^+/Ca^{2+} exchange in canine cardiac sarcolemmal vesicles. *Circ. Res.* 50:287–293.

41. Trosper, T. L., and K. D. Philipson. 1983. Effects of divalent and trivalent cations in Na/Ca exchange in cardiac sarcolemmal vesicles. *Biochim. Biophys. Acta.* 731:63–68.

42. Smith, J. B., E. J. Cragoe, Jr., and L. Smith. 1987. Na^+/Ca^{2+} antiport in cultured arterial smooth muscle cells. Inhibition by magnesium and other divalent metals. *J. Biol. Chem.* 264:831–837.

43. Lichtman, A. H., G. B. Segel, and M. A. Lichtman. 1981. Calcium transport and calcium-ATPase activity in human lymphocyte plasma membrane vesicles. *J. Biol. Chem.* 256:6148–6154.

44. Smith, J. B., S. D. Dwyer, and L. Smith. 1989. Decreasing extracellular Na^+ concentration triggers inositolpolyphosphate production and Ca^{2+} mobilization. *J. Biol. Chem.* 264:831–837.

45. Dolmetsch, R. E., and R. S. Lewis. 1994. Signaling between intracellular Ca^{2+} stores and depletion-activated Ca^{2+} channels generates $[Ca^{2+}]_i$ oscillations in T lymphocytes. *J. Gen. Phys.* 103:365–388.

46. Donnadieu, E., D. Cefai, Y. P. Tan, G. Paresys, G. Bismuth, and A. Trautmann. 1992. Imaging early steps of human T cell activation by antigen-presenting cells. *J. Immunol.* 148:2643–2653.

47. Wacholtz, M. C., and P. E. Lipsky. 1993. Anti-CD3-stimulated Ca^{2+} signal in individual human peripheral T cells. *J. Immunol.* 150:5338–5349.

48. Scharff, O., B. Foder, O., Thastrup, B. Hofmann, J. Moller, L. P. Ryder, K. D. Jacobsen, E. Langhoff, E. Dickmeiss, S. B. Christensen, P. Skinhoj, and A. Svejgaard. 1988. Effect of thapsigargin on cytoplasmic Ca^{2+} and proliferation of human lymphocytes in relation to AIDS. *Biochim. Biophys. Acta.* 972:257–264.

49. Breittmayer, J.-P., M. Ticchioni, B. Ferrua, A. Bernard, and C. Aussel. 1993. Ca^{2+}-ATPase inhibitors induce interleuken-2 synthesis and T cell proliferation. *Cell. Immunol.* 149:248–257.

50. Imboden, J. B., and A. Weiss. 1987. The T-cell antigen receptor regulates sustained increases in cytoplasmic free Ca^{2+} through extracellular Ca^{2+} influx and ongoing intracellular Ca^{2+} mobilization. *Biochem. J.* 247:695–700.

51. Premack, B. A., and P. Gardner. 1992. Signal transduction by T-cell receptors: mobilization of Ca^{2+} and regulation of Ca-dependent effector molecules. *Am. J. Physiol.* 263(*Cell Physiol. 32*):C1119–C1140.

52. Rosskopf, D., E. Frömter, and W. Siffert. 1993. Hypertensive sodium-proton exchanger phenotype persists in immortalized lymphoblasts from essential hypertensive patients. A cell culture model for human hypertension. *J. Clin. Invest.* 92:2553–2559.

53. Reusch, H. P., R. Reusch, D. Rosskopf, W. Siffert, J. F. E. Mann, and F. C. Luft. 1993. Na^+/H^+ exchange in human lymphocytes and platelets in chronic and subacute metabolic acidosis. *J. Clin. Invest.* 92:858–865.