The Lymphocyte as a Cellular Model to Study Insights into the Pathophysiology of Diabetes and Its Complications

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ABSTRACT: Blood cells from subjects with hypertension and/or diabetes mellitus have been successfully studied in the past to gain insight into pathological alterations of several signal transduction pathways. Diabetes mellitus is also considered to be a disease of abnormal cellular Ca^{2+} metabolism, as metabolic derangements of Ca^{2+} transport have been noticed both in the prediabetic state and as a consequence of hyperglycemia and oxidative stress. In this report, we used peripheral blood lymphocytes from type 2 diabetes patients and control subjects to study and delineate different mechanisms of Ca^{2+} turnover that determine the level of cytosolic calcium (Ca_i). While demonstrating the specific Ca^{2+} turnover alterations, we suggest that insights into the pathophysiology of diabetic complications originating from signal transduction defects could be conveniently studied using blood cell types such as lymphocytes and that such studies could lead to the identification of new molecular drug targets.

KEYWORDS: diabetes; cytosolic calcium; lymphocytes; SERCA, PMCA, PKC, signal transduction

INTRODUCTION

Cellular signaling defects are considered to play a fundamental role in the development of diabetes and diabetes-associated complications. Blood cells from subjects exhibiting diabetes mellitus have been successfully studied in the past to gain insight into pathological alterations of several signal transduction pathways. Particularly, the measurements of ion transport turnover in lymphocytes (and platelets) have been shown to be highly reproducible and even persistent in cultured transformed cells.[1] It is inferred from our work[2,3] and studies elsewhere[1] that using lymphocytes as a cellular paradigm has several advantages: (*a*) Lymphocytes are an easily accessible blood cell type and it is convenient to obtain clinical samples. (*b*) Lymphocyte Ca^{2+} signaling aspects and molecular players of Ca^{2+} regulation are well understood. (*c*) Lymphocytes contain the same transport proteins present in vascular smooth muscle cells, myocardial tissue, and other target cells affected in diabetes mellitus. (*d*) Interesting analogies exist in ion channel plasticity between lymphocytes and excitable

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Ann. N.Y. Acad. Sci. 958: 399–402 (2002).

cells. (*e*) Immortalized lymphoblasts conserve "diabetic" phenotype characteristics of ionic alterations. (*f*) Lymphocytes could potentially provide genomic DNA for studying genetic mechanisms of diabetic complications.

Altered cellular and subcellular Ca^{2+} distribution along with augmentation of Na^+/H^+ antiport and/or protein kinase C (PKC) could impose long-lasting effects on diabetes and its micro- and macrovascular complications. In this report we summarize some of our work on Ca^{2+} turnover and briefly discuss the evidence that supports the use of lymphocytes as a suitable cellular model to study the pathogenic signal transduction mechanisms of diabetes and its complications.

METHODS

Peripheral lymphocytes were isolated from whole blood using Lymphoprep and centrifugation. Measurements of intracellular calcium (Ca_i), ATPase activity, Na^+/Ca^{2+} exchange, and thapsigarin (Tg)-mediated Ca^{2+} influx were performed as per our previous protocols.[2,3] Age- and weight-matched control and type 2 diabetes subjects were recruited from the M.V. Diabetes Specialities Center, Chennai.

RESULTS AND DISCUSSION

It appears that several aspects of lymphocyte Ca^{2+} transport and regulatory profiles are similar to those in vascular tissues and β cells. We have used thapsigargin to monitor changes in lymphocyte Ca^{2+} fluxes. Thapsigargin (Tg), which is a specific inhibitor of sarcoendoplasmic calcium ATPase (SERCA),[4] induces Ca^{2+} entry into cells from the extracellular space as a result of depletion of Ca^{2+} from the intracellular stores. As illustrated in FIGURE 1, cells treated with Tg exhibited dose-dependent increments in Ca_i in the range of 75 to 400 nM. The Ca_i profile of cells exposed to Tg demonstrated two phases: a slow increase in Ca_i lasting 50 to 100 seconds, followed by a steep rise in the Ca_i. Each of these traces in this figure was de-

TABLE 1. Cellular (lymphocyte) phenotypic characteristics of Ca^{2+} turnover

	Control subjects (n = 12)	Type 2 diabetes (n = 12)
Basal Ca_i (nM)	43.7 ± 2.6	57.4 ± 3.7*
Plasma membrane Ca^{2+}-ATPase (PMCA) activity (μmol/mg protein/min)	0.110 ± 0.008	0.073 ± 0.007*
Reverse mode Na^+/Ca^{2+} exchange activity (-fold increase over basal after Tg treatment)	<1	3
Tg-stimulated $^{45}Ca^{2+}$ uptake (%)	23.2 ± 3.7	47.8 ± 14.5*
Tg-stimulated initial peak Ca_i (nM)	162 ± 11	235 ± 21*
PKC activation–related inhibition of Tg-mediated Ca^{2+} influx (%)	68 ± 2.6	38 ± 2.9*

*$P < 0.05$.

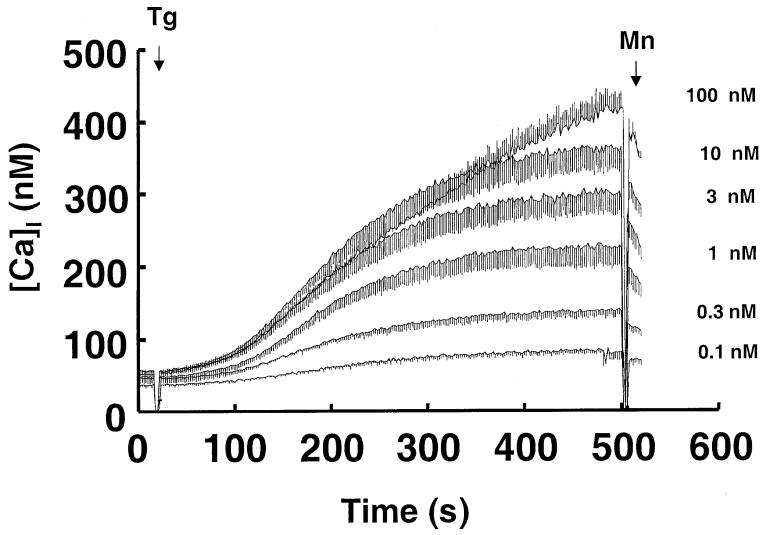

FIGURE 1. Dose-dependent thapsigargin (Tg)–induced Ca^{2+} entry in lymphocytes from control subjects. Lymphocytes were loaded with fura 2-AM for 30 min in Hepes buffered saline (HBS) containing 1 mM $CaCl_2$. Prior to each experiment, cells were centrifuged for 5 s, resuspended in 100 μL HBS, and injected into cuvettes containing 3 mL of solution. Ca_i was monitored at 37 °C with constant stirring in a spectrofluorimeter. Tg (0.1nM to 100nM) was added at 20 s, and the Ca^{2+} profile was monitored for 500 s, after which $MnCl_2$ was added to assess dye leakage. Each dose-dependency trace was derived from the mean of six independent experiments.

rived from mean fluorescence values of six different sets of experiments and this confirms the accuracy and reproducibility of Ca_i measurements. TABLE 1 summarizes the results of Ca^{2+} turnover parameters in cells from type 2 diabetes and control subjects as a result of fluorescence and $^{45}Ca^{2+}$ flux studies. As evident from the table, cells from type 2 diabetes exhibited significantly higher ($P < 0.05$) basal Ca_i, reduced plasma membrane calcium ATPase activity, and higher Na^+/Ca^{2+} exchange and Tg-stimulated Ca^{2+} fluxes. In cells from type 2 diabetes, the PKC-activation related feedback inhibition of Ca^{2+} influx was noticed to be considerably reduced. The higher basal Ca_i resulting from altered Ca^{2+} transport in cells from type 2 diabetes also exhibited a positive correlation with the plasma glucose (control 79 ± 11 vs. NIDDM 176 ± 27 mg/dL) and glycated hemoglobin (HbA1c) values (control; 5.7 ± 0.5 vs. NIDDM 9.0 ± 1.7 %)

It has been proposed that abnormal cell Ca^{2+} homeostasis may contribute to the impaired insulin action because part of the insulin action may be mediated by the Ca^{2+} signal.[5] As multiple cell functions are regulated by Ca_i- and Ca^{2+}-dependent signals, it is conceivable that altered refilling of the intracellular Ca^{2+} pools resulting from modulatory patterns of the store-operated Ca^{2+} entry may translate into dysregulated protein synthesis and proliferation. In patients with mitochondrial DNA inherited oxidative phosphorylation deficiency, derangement of organellar Ca^{2+} handling has been reported which impairs the Ca^{2+}-mediated activation of mito-

chondrial activity.[6] Our preliminary studies in type 2 diabetes also indicate a defect in the organellar sequestration of Ca^{2+} especially in mitochondria and an increase in the cytosolic reactive oxygen species (ROS) load. Restoration of Ca^{2+} signal and the enhancement of ATP production in mitochondria could correct insulin secretory and sensitizing effects in maturity-onset diabetes in young (MODY) and common forms of type 2 diabetes subjects.

Our studies suggest that in the absence of target tissues amenable for cellular and molecular studies, insights into the pathophysiology of diabetic complications originating from signal transduction defects could be conveniently studied using blood cell types such as lymphocytes. Mononuclear cells better serve to explore the role and causal relationship of intracellular ions (e.g., Ca^{2+}, H^+, Na^+), cytosolic messenger molecules, cytokines, Th1 and Th2 signals, transcription factors, and gene expressions in the development and progression of micro- and macrovascular complications of diabetes. It seems that their use in clinical diabetes would also identify whether the amount of diabetes-induced intracellular oxidative stress (reactive oxygen species [ROS] load) or the concentration of hyperglycemia-induced advanced glycation end products is associated with the risk of diabetic complications.[7] We predict that the experimental approach of immortalized blood cells from patients with diabetes and diabetic complications could potentially open up new horizons in the identification of genetic abnormalities in intracellular signal transduction.

REFERENCES

1. SIFFERT, W. & R. DUSING. 1996. Na^+/H^+ exchange in hypertension and in diabetes mellitus: facts and hypotheses. Basic Res. Cardiol. **91:** 179–190.
2. BALASUBRAMANYAM, M., C. ROHOWSKY-COCHAN, J.P. REEVES & J.P. GARDNER. 1994. Na^+/Ca^{2+} exchange-mediated calcium entry in human peripheral blood lymphocytes. J. Clin. Invest. **94:** 2002–2008.
3. BALASUBRAMANYAM, M., A. BALAJI, B. SUBASHINI & V. MOHAN. 2001. Evidence for mechanistic alterations of Ca^{2+} homeostasis in type 2 diabetes mellitus. Int. J. Exp. Diab. Res. **1:** 275–287.
4. THASTRUP, O., P.J. CULLEN, B.K. DROBAK, *et al.* 1990. Thapsigargin, a tumor promotor, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. Proc. Natl. Acad. Sci. USA **87:** 2466–2470.
5. LEVY, J. 1999. Abnormal cell calcium homeostasis in Type 2 diabetes mellitus: a new look on old disease. Endocrine **10:** 1–6.
6. BRINI, M., P. PINTON, M.P. KING, *et al.* 1999. A calcium signaling defect in the pathogenesis of mitochondrial DNA inherited oxidative phosphorylation deficiency. Nat. Med. **5:** 951–954.
7. HAMMES, H.P., M. BROWNLEE, J. LIN, *et al.* 1999. Diabetic retinopathy risk correlates with intracellular concentrations of the glycoxidation product N-epsilon-(carboxymethyl) lysine independently of glycohaemoglobin concentration. Diabetologia **42:** 603–607.