European Journal of Pharmaceutical Sciences 44 (2011) 602–609

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Lysophosphatidylcholine induces Ca^{2+} mobilization in Jurkat human T lymphocytes and CTLL-2 mouse T lymphocytes by different pathways

Qi Wang, Yi-Jun Wu *

Laboratory of Molecular Toxicology, State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, PR China

ARTICLE INFO

Article history:
Received 31 July 2011
Received in revised form 4 October 2011
Accepted 7 October 2011
Available online 14 October 2011

Keywords:
Lysophosphatidylcholine
Lymphocyte
Intracellular calcium concentration
L-type calcium channel
Ryanodine receptor

ABSTRACT

Lysophosphatidylcholine (LPC), an important compound in the immune system, regulates a variety of biological processes. We examined and compared the effect of exogenous LPC on intracellular Ca^{2+} overload in human Jurkat CD4+ T lymphocytes and mouse CTLL-2 CD8+ T lymphocytes. LPC caused a dose-dependent intracellular Ca^{2+} level ($[Ca^{2+}]_i$) increase in both Jurkat and CTLL-2 lymphocytes. Pretreatment of cells for 5 min with 30 μM of ruthenium red, a potent ryanodine receptor inhibitor, reduced the LPC-induced Ca^{2+} response in both Jurkat and CTLL-2 T lymphocytes. Moreover, pretreatment of cells with 100 μM 2-APB for 15 min, a cell-permanent IP_3 receptor inhibitor, reduced about two thirds of the LPC induced calcium response in both kinds of cells. However, preincubation of the cells with verapamil, an L-type Ca^{2+} channel blocker, did not affect the LPC-induced $[Ca^{2+}]_i$ increase in CTLL-2 lymphocytes but inhibited this in Jurkat lymphocytes by 26%. In Ca^{2+}-free medium, LPC produced 75.8% of the total $[Ca^{2+}]_i$ increase in CTLL-2 lymphocytes and 38% of the total $[Ca^{2+}]_i$ increase in Jurkat lymphocytes. These data suggested that the LPC-induced $[Ca^{2+}]_i$ increase in human Jurkat and mouse CTLL-2 cell lines occurs via different pathways.

1. Introduction

Lysophosphatidylcholine (LPC) is an intrinsic, intracellular messenger generated by the hydrolysis of membrane phosphatidylcholine by intracellular phospholipase A2 (PLA2). It regulates a variety of biological processes including cell proliferation, differentiation, tumor cell invasiveness, and inflammation (Spiegel and Milstien, 1995; Moolenaar, 1999). In the immune system, LPC promotes inflammatory effects, including monocyte chemotaxis, macrophage activation, and inducement of apoptosis in normal, activated lymphocytes (Jing et al., 2000). As a component of oxidized low density lipoprotein, LPC plays an etiological role in atherosclerosis and is implicated in the pathogenesis of the autoimmune disease, systemic lupus erythematosus (SLE) (Koh et al., 2000). LPC is also an important natural adjuvant for the immune system, inducing humoral and cellular immune responses (Perrin-Cocon et al., 2006). Most important of all, as determined by the expression of a subunit of the interleukin 2 receptor and thymidine incorporation into DNA (Asaoka et al., 1992), LPC greatly potentiates the activation of human resting T lymphocytes that is induced by a membrane permeant diacylglycerol plus a calcium ionophore.

Recently, an orphan G-protein-coupled receptor (GPCR), G2A, has been identified as a high-affinity receptor for LPC and is expressed predominantly in lymphoid tissues and lymphocytes (Kabarowski et al., 2001). G2A was originally isolated as a stress-inducible GPCR that induces the cell cycle arrest at G2/M periods when cells are serum-starved or DNA-damaged; its expression is induced by cellular activation and stress (Weng et al., 1998). LPC, interacting with its receptor, G2A, induces the elevation of the intracellular calcium concentration ($[Ca^{2+}]_i$) and also the transcriptional activation of the serum response factor via the MAPK pathway (Murakami et al., 2004).

Calcium ions (Ca^{2+}) function as a universal second messenger in virtually all eukaryotic cells, including those of the immune system. The elevation of intracellular free Ca^{2+} is an essential trigger for T cell differentiation (Crabtree, 1989) and activation (Weiss and Imboden, 1987; Premack and Gardner, 1992; Feske, 2007). The engagement of T cell receptors and the rise in $[Ca^{2+}]_i$ is followed by a number of downstream effects. Changes in motility and cytoskeletal reorganization occur soon after T cells contact antigen-presenting cells (Gelfand, 1990; Donnadieu et al., 1994).

Abbreviations: 2-APB, 2-aminoethoxydiphenyl borate; CRAC, calcium release-activated calcium current; FBS, fetal bovine serum; GPCR, G-protein-coupled receptor; IP_3, inositol trisphosphate; LPC, lysophosphatidylcholine; PLA2, phospholipase A2; SCID, severe combined immunodeficient disease; SLE, systemic lupus erythematosus; WAS, Wiskott–Aldrich syndrome.

* Corresponding author. Address: Institute of Zoology, Chinese Academy of Sciences, 1-5 Beichenxi Road, Chaoyang District, Beijing 100101, PR China. Tel.: +86 10 64807251; fax: +86 10 64807099.

E-mail address: wuyj@ioz.ac.cn (Y.-J. Wu).

0928-0987/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejps.2011.10.007

Over periods of minutes to hours, the amplitude, duration, and kinetic signature of Ca^{2+} signals increase the efficiency and specificity of gene activation events, such as IL-2, a cytokine essential for T-cell proliferation and the immune response (Crabtree, 1989; Gelfand, 1990; Negulescu et al., 1994; Rao, 1994). Furthermore, cell killing by cytotoxic T cells (Poenie et al., 1987; Haverstick et al., 1991), apoptosis of self-reactive T cells during development in the thymus (McConkey et al., 1992; Nakayama et al., 1992), induction of tolerance to self-antigens in mature peripheral T cells (Schwartz, 1990; Nghiem et al., 1994) and inhibition on n-type K^+ channels in both T and B lymphocytes, also occur after the elevation of $[Ca^{2+}]_i$.

It has been reported that LPC induces several cellular changes in Jurkat lymphocytes, including an increase of ROS generation in a PKCδ-dependent and GPCR-independent manner, a marked and immediate tyrosine phosphorylation, a decrease of MMP, and most importantly, an increase of $[Ca^{2+}]_i$ through Ca^{2+} influx (Im et al., 2006). Legradi et al. reported that the LPC-induced $[Ca^{2+}]_i$ increase in Jurkat T cells was dependent on PTX-sensitive G proteins (Légrádi et al., 2004). Inhibition of the Ser/Thr kinases and tyrosine kinases with staurosporine and genistein, respectively, suppressed the rise in $[Ca^{2+}]_i$. Staurosporine, the inhibitor of Ser/Thr kinases impedes the LPC-induced $[Ca^{2+}]_i$ elevation. Furthermore, activation of G2A by LPC increased $[Ca^{2+}]_i$ induced receptor internalization, activated ERK mitogen-activated protein kinase and modified migratory responses of Jurkat T lymphocytes (Kabarowski et al., 2001).

Although LPC which is involved in the activation of T lymphocytes and apoptosis of activated lymphocytes can cause an increase in $[Ca^{2+}]_i$ that is also very significant for immune response, the exact signal pathway of LPC-induced $[Ca^{2+}]_i$ increase is poorly defined for T lymphocytes, especially for cytotoxic T lymphocytes. In this paper we examine and compare the pathways of exogenous LPC on intracellular Ca^{2+} overload in human Jurkat CD4+ T lymphocytes and mouse CTLL-2 CD8+ T lymphocytes. Using the two cell lines as models of helper and cytotoxic T lymphocytes, we may postulate the possible different mechanism in activation of two kinds of T lymphocytes in which LPC-induced the Ca^{2+} changes is involved.

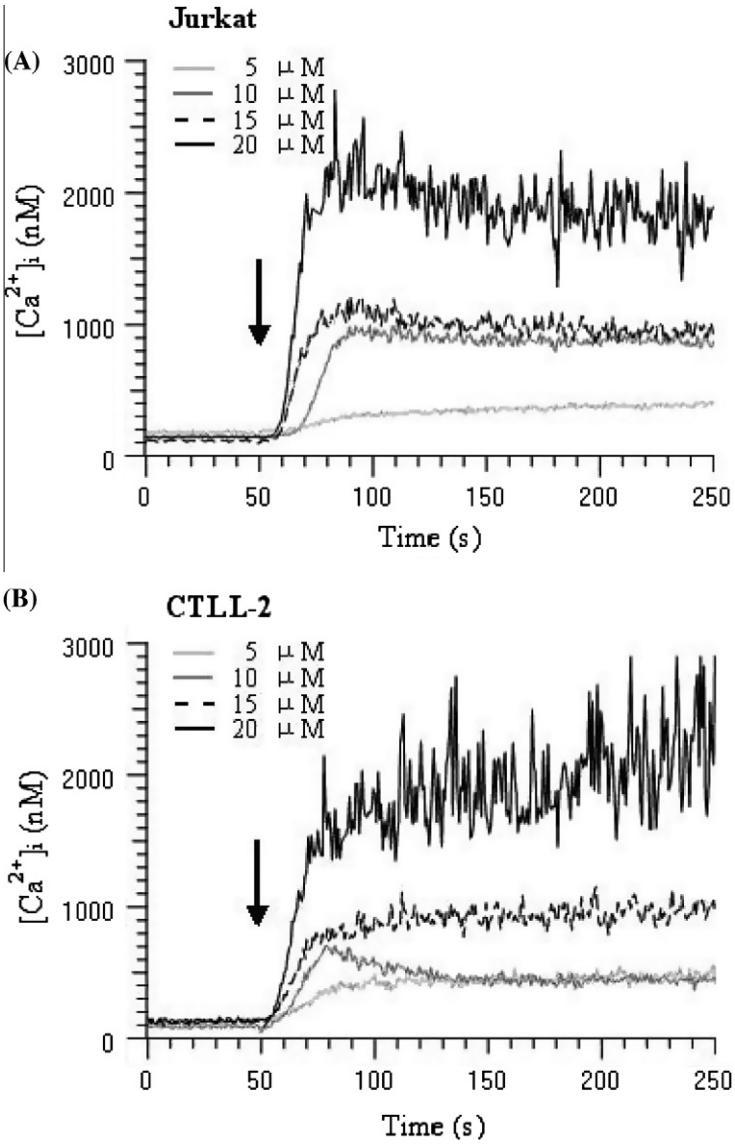

Fig. 1. Change in Ca^{2+} in response to different concentrations of LPC in Jurkat and CTLL-2 T lymphocytes. A (Jurkat) and B (CTLL-2): cells loaded with Fura-2/AM were stimulated by the addition of LPC in Ca^{2+}-containing medium. Arrows indicate the time of LPC addition. Traces were representative of at least 4 experiments.