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Characterization of lysophosphatidylcholine-induced changes of intracellular calcium in *Drosophila* S2 cells

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ABSTRACT

Aims: Lysophosphatidylcholine (LPC), a bioactive lipid, regulates a wide array of biological processes. LPC could be deacylated to form glycerophosphocholine by neuropathy target esterase (NTE)/Swiss cheese protein (SWS). Although NTE/SWS is important in maintaining Ca^{2+} homeostasis, the role of LPC in regulating the intracellular calcium concentration ($[Ca^{2+}]_i$) in *Drosophila* remains poorly understood. We aimed to study the mechanism of LPC-induced $[Ca^{2+}]_i$ changes in *Drosophila* S2 cells.
Main methods: The $[Ca^{2+}]_i$ of *Drosophila* S2 cells was measured by fluorescence spectrophotometry after loading the cells with the calcium-sensitive fluorescent probe Fura-2/AM.
Key findings: Our results demonstrated that LPC could cause a rapid, dose-dependent increase in the $[Ca^{2+}]_i$ in the presence of external calcium ($[Ca^{2+}]_e$). The LPC-induced $[Ca^{2+}]_i$ increase was reduced by 60.7% in the absence of $[Ca^{2+}]_e$. Furthermore, the Ca^{2+} influx was inhibited by 37.3% after the cells were preincubated with an L-type Ca^{2+} channel blocker. In the Ca^{2+}-free medium, the LPC-induced $[Ca^{2+}]_i$ increase was completely blocked using an inositol triphosphate receptor (IP_3R) inhibitor. However, a ryanodine receptor (RyR) inhibitor had no effect on the LPC-induced $[Ca^{2+}]_i$ increase.
Significance: The LPC-induced $[Ca^{2+}]_i$ increase in S2 cells was dependent on both the release of Ca^{2+} stored in the endoplasmic reticulum and $[Ca^{2+}]_e$ influx. Both L-type Ca^{2+} channels and IP_3R might be involved in this process. The LPC-induced $[Ca^{2+}]_i$ increase in S2 cells characterized in this study may shed light on the study of NTE/SWS protein function in general because the enzyme is responsible for the deacylation of LPC.

1. Introduction

Lysophosphatidylcholine (LPC) mediates a number of physiological functions and regulates a wide array of biological processes [10,25]. LPC is regularly produced by the partial hydrolysis and deacylation of phosphatidylcholine, a major component of plasma membranes, by neuropathy target esterase (NTE), which could further deacylate LPC to form glycerophosphocholine [29,36]. In *Drosophila*, Swiss cheese protein (SWS) is the homologous protein of NTE [9]; SWS plays an important role in regulating brain development in insects [17]. Previous studies revealed that the intracellular calcium concentration ($[Ca^{2+}]_i$) decreased if NTE activity was inhibited [8,27], suggesting that NTE/SWS and LPC may be involved in the regulation of calcium homeostasis in *Drosophila*.

It is known that calcium ions (Ca^{2+}) are critical intracellular messengers responsible for regulating numerous cellular processes in both vertebrates and invertebrates [2–4]. For example, in *Drosophila*, calcium activates and regulates Cl^- channels in S2 cells [6] and is also critical for the maturation and function of neurons in the adult brain [15]. The transient increase in intracellular calcium changed gene expression and enhanced neurotransmitter release [5,33]. In *Drosophila* photoreceptors, extracellular calcium entered through a light-activated channel and regulated the phototransduction cascade [13,30].

Although NTE/SWS may be important in maintaining Ca^{2+} homeostasis, the role of LPC in intracellular calcium homeostasis in *Drosophila* remains poorly understood. In this paper, we studied the mechanism of $[Ca^{2+}]_i$ changes induced by LPC in *Drosophila* S2 cells, which were derived from embryonic cells of Drosophila melanogaster [31].

2. Materials and methods

2.1. Materials

Fura-2/AM, TNM-FH insect medium, verapamil, 2-aminoethoxydiphenyl borate (2-APB), L-a-lysophosphatidylcholine (primary palmitate and stearate esters), and ruthenium red were purchased from Sigma Aldrich Co. (St. Louis, MO, USA). *Drosophila* S2 cells (ATCC number: CRL-1963™) were kindly donated by Dr. Jun-Yong Huang

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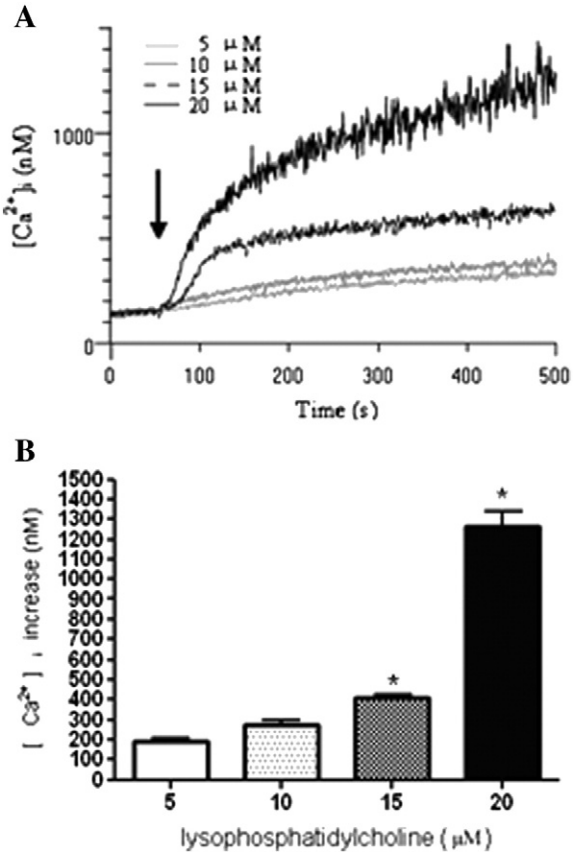

Fig. 1. Ca^{2+} levels in *Drosophila* S2 cells in response to different concentrations of LPC. A: Fura-2/AM-loaded cells were stimulated by the addition of LPC in Ca^{2+}-containing medium. The arrow indicates the time of LPC addition. The traces are representatives of at least 4 experiments. B: The average $[Ca^{2+}]_i$ increase above the basal level at different LPC concentrations. Data are the means ± SD from at least 4 separate experiments. $^{*}P < 0.05$.

(University of Newcastle, UK). Foetal bovine serum (FBS) was obtained from Chuanye Bioscience (Tianjing, China).

2.2. Cell culture

Drosophila S2 cells were cultured with TNM-FH insect medium supplemented with 10% heat-inactivated FBS plus 100 μg/ml streptomycin and 100 units/ml penicillin. Cells were grown in a monolayer at 23 °C and passaged every three days. Cultures with 80% confluence were used in all subsequent experiments.

2.3. Measurement of the intracellular calcium concentration

The $[Ca^{2+}]_i$ was measured using the calcium-sensitive fluorescent indicator Fura-2/AM. The cells were washed, resuspended in HEPES-buffered medium (HBM) (120 mM NaCl, 32 mM sucrose, 10 mM HEPES, 8 mM $MgCl_2$, 5 mM KCl, and 2 mM $CaCl_2$, pH 7.2) at 1×10^7 cells/ml, and then incubated at 37 °C with 2.5 μM Fura-2/AM for 20 min. The cells were resuspended in the same HBM at a density of 1×10^6 cells/ml after being washed twice. Fluorescence intensity was monitored at 510 nm (5 nm slit) using alternating excitation at 340 nm and 380 nm (5 nm slit) with a F4500 dual wavelength fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

The $[Ca^{2+}]_i$ was calculated based on the equation in Grynkiewicz et al. [12], in which a dissociation constant (Kd) of 224 nM [20] was used. Intracellular Cation Scan software (FL Solutions 2.0; Lambda Advanced Technology, UK) was used. The minimum and maximum fluorescence intensity values were obtained by the sequential addition of 5 mM EGTA and 0.1% Triton X-100.

In Ca^{2+}-free experiments, Fura-2/AM-loaded cells were washed and then suspended in Ca^{2+}-free medium for fluorescence measurement. In Ca^{2+} restoration experiments, the initial Ca^{2+}-free medium was replaced with 2 mM Ca^{2+} 100 s after treatment with LPC.

2.4. Treatment of Drosophila S2 cells with different inhibitors

Fura-2/AM-loaded cells were pretreated for 15 min with 20 μM verapamil (an L-type Ca^{2+} channel blocker) or 10 μM/100 μM 2-APB (an inhibitor of the inositol trisphosphate receptor, IP_3R). In another set of experiments, the cells were pretreated for 5 min with 30 μM ruthenium red (a ryanodine receptor (RyR) inhibitor). Treated cells were subsequently stimulated with 15 μM LPC.

2.5. Statistical analysis

The means of three or more treatment groups were compared by ANOVA and Fischer's LSD test, and the means of two treatment groups were compared using unpaired Student's two-tailed t tests. P-values <0.05 were considered significantly different. All data are reported as the means ± standard deviation (SD).

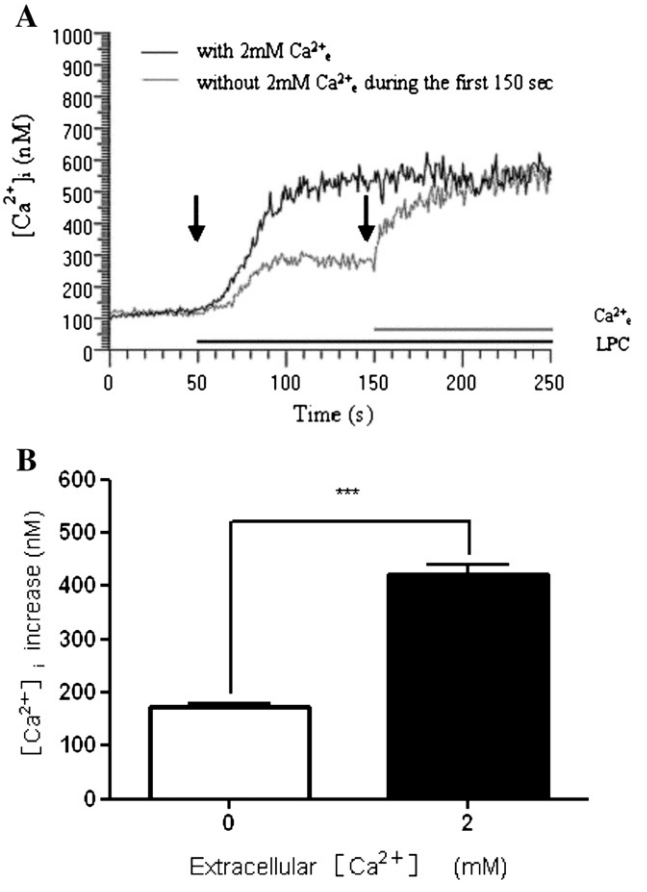

Fig. 2. The effect of external Ca^{2+} on the LPC-induced Ca^{2+} response in *Drosophila* S2 cells. Fura-2/AM-loaded cells were stimulated by the addition of 15 μM LPC in the presence or absence of extracellular Ca^{2+} in the medium. A: Typical traces of $[Ca^{2+}]_i$ induced by LPC with 2 mM extracellular Ca^{2+} either present (black) or absent (grey) during the first 150 s. Ca^{2+} was then restored to 2 mM (second arrow) in Ca^{2+}-free medium 100 s after LPC stimulation (first arrow). The solid bars indicate the presence of LPC (black) and Ca^{2+} (grey). The traces are representatives of at least 3 experiments. B: The average $[Ca^{2+}]_i$ increase above the basal level in the presence (■) or absence (□) of external Ca^{2+}. Data are the means ± SD from at least 3 separate experiments. $^{***}P < 0.01$.