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Lysophospholipids modulate voltage-gated calcium channel currents in pituitary cells; effects of lipid stress

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ABSTRACT

Voltage-gated calcium channels (VGCCs) are osmosensitive. The hypothesis that this property of VGCCs stems from their susceptibility to alterations in the mechanical properties of the bilayer was tested on VGCCs in pituitary cells using cone-shaped lysophospholipids (LPLs) to perturb bilayer lipid stress. LPLs of different head group size and charge were used: lysophosphatidylcholine (LPC), lysophosphatidylinositol (LPI), lysophosphatidylserine (LPS) and lysophosphatidylethanolamine (LPE). Phosphatidylcholine (PC) and LPC (C6:0) were used as controls. We show that partition of both LPC and LPI into the membrane of pituitary cells suppressed L-type calcium channel currents (I_L). This suppression of I_L was slow in onset, reversible upon washout with BSA and associated with a depolarizing shift in activation (~8 mV). In contrast to these effects of LPC and LPI on I_L, LPS, LPE, PC and LPC (C6:0) exerted minimal or insignificant effects. This difference may be attributed to the prominent conical shape of LPC and LPI compared to the shapes of LPS and LPE (which have smaller headgroups), and to PC (which is cylindrical). The similar effects of LPC and LPI on I_L, despite differences in the structure and charge of their headgroups suggest a common lipid stress dependent mechanism in their action on VGCCs.

1. Introduction

Voltage-gated calcium channels (VGCCs) in pituitary cells – key players in the secretion of pituitary hormones [1,2] – are regulated by releasing-hormones, neurotransmitters and transduction pathways, either via effects on membrane potential, or directly through effects on the channel proteins themselves [3]. They are also regulated by alterations in osmotic stress and mechanical membrane tension. L-type and T-type calcium channel currents (I_L and I_T, respectively) in pituitary cells are modulated by alterations in osmotic stress; osmotic shrinkage suppresses whereas osmotic swelling enhances I_L and I_T [4,5]. These osmotic effects on I_L and I_T persist after disruption or stabilization of cortical actin. A plausible explanation for this osmosensitivity is that VGCCs in pituitary cells are mechanosensitive; i.e. that membrane deformations transmitted via the lipid bilayer alters calcium channel function. Indeed osmo/mechanosensitivity has been demonstrated for VGCCs in smooth muscle cells [6] and in cardiac myocytes [7]. Furthermore, extensive recent studies demonstrated that recombinant VGCCs [8,9] and other voltage-gated channels (VGCs), respond to membrane stretch (thoroughly reviewed in [10]). This inherent responsiveness of VGCs to bilayer deformations reflects the intimate and extensive contact between VGCs amino acid residues and the highly structured (energetically speaking) lipid profile across the bilayer, and would explain the broad susceptibility of VGCs to structural perturbations of the bilayer produced by insertion of amphiphiles [10–13].

In this study we examined whether perturbations in the lipid bilayer, produced by insertion of cone-shaped amphiphiles into membranes of pituitary cells, alters VGCC function. Amphiphiles were previously defined by their molecular shape as cones, inverted-cones or cylinders, depending on the relative size of their polar head and hydrophobic tail [14]. Lipids consisting of a large polar head and a thin hydrophobic tail were defined as cones. It was proposed that partition of cone-shaped amphiphiles into the outer leaflet of the bilayer deforms membranes, increasing bilayer spontaneous curvature [15,16] and altering the mechanical properties of the bilayer [12,13,17]. As cone-shaped amphiphiles we have used lysophospholipids (LPLs) that differ in the size and polarity of their headgroups; LPC, LPI, LPE and LPS. These LPLs were previously used to investigate mechanosensitive gating of gramicidin channels [17], MScL channels [18], TREK-1 and TRAAK channels [19,20] and NMDA receptors [21]. Additional studies demonstrated mixed effects of LPC on ionic currents; LPC augmented HERG K^+ currents [22] and TRPC5 currents [23], and suppressed calcium currents [24] and sodium currents [25].

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We show here that LPC suppressed I_L, and produced a positive voltage shift in the activation of I_L. These effects of LPC were mimicked by LPI but not by LPE and LPS, suggesting that the prominent conical shape of LPC and LPI is a determinant in their action on I_L. We conclude that perturbation in the lipid bilayer, produced by partition of cone-shaped lysophospholipids, alters the mechanical properties of the bilayer, thereby affecting the normal function of VGCCs in pituitary cells.

2. Materials and methods

2.1. Cell culture

2.1.1. Primary pituitary cells

Animals were sacrificed in accordance with the Guidelines of the Authority for Animal Facilities-Ethics committee, Hebrew-University. Anterior pituitary glands were dissected from 3 male rats (Sabra strain 250–300 g). The isolated anterior lobes were then subjected to enzymatic dissociation in a procedure which was previously described in detail [4,5]. Enriched populations of somatotrophs and lactotrophs were obtained by using a 3-step discontinuous percoll gradient. Cells were plated onto round 16 mm glass coverslips and kept in the incubator (37 °C, 5% CO_2) for 1–6 days. Coverslips containing pituitary cells were placed in a perfusion chamber (RC-40LP, Warner Inst. CT, USA), and continuously perfused at a rate of ~1 ml/min at room temperature (20–24 °C).

2.1.2. Pituitary GH_3 cells

Pituitary GH_3 cells (purchased from ATCC, USA) were grown as monolayer in plastic culture flasks (Nunc, Denmark) containing DMEM supplemented with 10% FCS, 1% L-glutamine and 1% penicillin–streptomycin (Beth-Haemek, Israel). The culture medium in the flasks was changed every 2–3 days. GH_3 cells were harvested during the logarithmic phase of growth, when they were visibly confluent in the flask. Harvesting of cells was achieved by short exposures (~1 min) to DMEM supplemented with 0.25% trypsin and 0.53 mM EDTA (Beth-Haemek, Israel). Harvested cells were then plated onto round 16 mm cover slips coated with polylysine 10 μg/ml (Sigma, USA) and placed in 35 mm culture dishes containing DMEM supplemented with 10% FCS (Beth-Haemek, Israel), 1–3 days before performance of electrophysiological recording. The flasks and the dishes were kept in the humidified incubator in 37 °C, 5% CO_2.

2.2. Electrophysiological recording and analysis

Whole-cell Ba^{2+} currents (I_{Ba}) were recorded with an Axopatch 1C amplifier (Axon Inst, USA), sampled at 10 kHz (Digidata 1320A, Axon Inst, USA) and filtered at 1 kHz with a four-pole low pass Bessel filter as previously described in detail [4,5]. Final access resistance was usually <15 MΩ. Linear leak currents were digitally subtracted using P/2 pulse protocols. The pclamp8 suit of programs (Axon Inst., USA) was used for on-line acquisition and for off-line analysis of membrane currents. I_{Ba} through I_L and I_T were usually activated with 200 ms voltage steps (interval; 10 s) from a holding potential (Vh) of −80 mV to various test potentials (Vt). *I–V* relationships of I_{Ba} were obtained either by using discrete voltage steps (−60 to +40 mV, ΔV = 10 mV) or by using voltage ramps ranging from −100 to +80 mV (duration 300 ms; interval 10 s). The usage of voltage ramp enabled both useful fast monitoring of changes in *I–V* relationships and useful separation between T-type and L-type currents. In some experiments double-pulse protocols were used to activate simultaneously I_T ($Vt \cong -30$ mV) and I_L ($Vt \cong 0$ mV). I_T, whenever present, was measured as peak I_{Ba} whereas I_L was measured either from the peak or from the sustained component of I_{Ba} (see Fig. 1). I_L showed very little inactivation (at 200 ms). Therefore, the sustained component of I_{Ba} gave a good and clean estimate of I_L (I_T fully inactivated). Activation curves of I_{Ba} were fitted to a Boltzmann function in the form of: $G/Gmax = 1/\{1 + \exp(-[(V_t - V_{0.5})/k])\}$ where G is barium conductance at different test potentials, Gmax is the maximal barium conductance, $V_{0.5}$ is the voltage at which $G/Gmax = 0.5$ (half activation) and k is the slope factor. $V_{0.5}$ values for *I–V* curves that were activated by voltage ramps were obtained by interpolation from the corresponding normalized *I*/Imax curves. Results are always reported as mean ± SE. Statistical analysis was performed using either Student's *t*-test or one-way ANOVA. *p*-Values of $p < 0.05$ were considered significant.

2.3. Solutions

The 'extracellular' (bath) solution contained (in mM): 150 TEA-Cl, 10 $BaCl_2$, 10 glucose and 10 HEPES (adjusted to pH = 7.3 with TEA (OH), osmolarity 300 mosmol). The 'intracellular' (pipette) solution contained (in mM): 138 CsCl, 1 $CaCl_2$, 11 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), 10 HEPES, 2 MgATP, 0.16 guanosine triphosphate (GTP) (adjusted to pH = 7.3 with Cs(OH), osmolarity; 300 mosmol). For whole-cell experiments with GH_3 cells the pipette solution contained (in mM): 138 CsOH, 138 L-aspartic acid, 1 $CaCl_2$, 11 EGTA, 10 HEPES, 2 MgATP, 0.16 GTP (adjusted to pH = 7.3 with Cs(OH), osmolarity; 300 mosmol). Osmolarity was measured with a vapor pressure osmometer; VAPRO 5520 (WESCOR, USA). In some experiments GTP was replaced with $GDP_\beta S$ (2–5 mM). In some other experiments the PKC inhibitor GF 109203X (5 μM) was added to the bath and pipette solutions. All chemicals were obtained from Sigma (St. Louis, MO, USA), except GF 109203X (A.G. Scientific, San Diego, CA, USA). The PKC inhibitor was dissolved in DMSO (final concentration 0.02%).

2.4. Lysophospholipids (LPLs)

Lysophosphatidylcholine (LPC), Lysophosphatidylinositol (LPI) and short chain LPC (C6:0) were dissolved in double-distilled water (DDW) to form stock solutions of 12.5, 15 and 12.5 mM, respectively. Lysophosphatidylethanolamine (LPE) was dissolved either in DDW or in ethanol to form stock solutions of 10 mM. LPC (egg), contained primarily saturated fatty acids (~96%) at the C1 position of the glycerol backbone; ~69% palmitic acid (C16:0) and ~27% stearic acid (C18:0) as acyl chains (TLC analysis, Sigma). Similarly, LPI (soybean) and LPE (*E. coli*) contained primarily palmitic and stearic acids as acyl chains. Lysophosphatidylserine (LPS-synthetic, oleoyl (C18:1) as acyl chain) was dissolved in ethanol to form a stock solution of 7 mM. The dissolution of both LPE and LPS required long lasting sonications at 4 °C, and final ethanol concentration in both LPE and LPS experiments was less than 0.1%. Phosphatidylcholine (PC-egg) was dissolved in ethanol and sonicated to prevent micelle formation. Final ethanol concentration in the bath was 0.02%. Washout of amphiphiles was performed with bovine albumin-essentially fatty acid free (0.5 mg/ml). All amphiphiles were obtained from Sigma (St. Louis, MO, USA) except LPS (Avanti, AL, USA).

3. Results

3.1. Differential effects of LPC on I_L and I_T

Pituitary cells were exposed to LPC (3–30 μM) for ~300 s. In some experiments, however, longer exposures to LPC were needed to get a significant response; up to 600 s. Fig. 1 demonstrates LPC-induced suppression of I_L and I_T. The onset of suppression