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Effect of surface-potential modulators on the opening of lipid pores in liposomal and mitochondrial inner membranes induced by palmitate and calcium ions

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ABSTRACT

The effect of surface-potential modulators on palmitate/Ca^{2+}-induced formation of lipid pores was studied in liposomal and inner mitochondrial membranes. Pore formation was monitored by sulforhodamine B release from liposomes and swelling of mitochondria. ζ-potential in liposomes was determined from electrophoretic mobility. Replacement of sucrose as the osmotic agent with KCl decreased negative ζ-potential in liposomes and increased resistance of both mitochondria and liposomes to the pore inducers, palmitic acid, and Ca^{2+}. Micromolar Mg^{2+} also inhibited palmitate/Ca^{2+}-induced permeabilization of liposomes. The rate of palmitate/Ca^{2+}-induced, cyclosporin A-insensitive swelling of mitochondria increased 22% upon increasing pH from 7.0 to 7.8. At below the critical micelle concentration, the cationic detergent cetyltrimethylammonium bromide (10 μM) and the anionic surfactant sodium dodecylsulfate (10–50 μM) made the ζ-potential less and more negative, respectively, and inhibited and stimulated opening of mitochondrial palmitate/Ca^{2+}-induced lipid pores. Taken together, the findings indicate that surface potential regulates palmitate/Ca^{2+}-induced lipid pore opening.

1. Introduction

Free fatty acids exert many biological effects, many involving mitochondria. Fatty acids are substrates for mitochondrial respiration, uncouplers of oxidative phosphorylation, inducers of the mitochondrial permeability transition (MPT) pore and pro-apoptotic agents [1–5]. In the presence of Ca^{2+}, long-chain saturated fatty acids also open a cyclosporin A (CsA)-insensitive pore in the mitochondrial inner membrane [6, 7]. Palmitate/Ca^{2+}also induces pores in erythrocyte membranes, artificial lipid vesicles, and black lipid membranes [7–11]. These findings indicate that the fatty acid/Ca^{2+}-induced pore is lipid in nature. The mechanism of formation of these lipidic pores is suggested to be high affinity binding of long-chain saturated fatty acids to Ca^{2+} with segregation of the fatty acid/Ca^{2+} complexes into pore-forming solid-crystalline membrane domains [11–13].

Albumin, which binds free fatty acids, and EGTA, a Ca^{2+} chelator, suppress the formation of lipid pores in liver mitochondria, whereas blockers of the MPT such as CsA have no effect on opening of palmitate/Ca^{2+}-induced pores in mitochondrial membranes [6, 9]. The physical–chemical properties of a lipid membrane, in particular, its phase state, depend on a number of factors: temperature, pressure, Ca^{2+}, and various small molecules, including fatty acids, that interact with the bilayer-forming lipid [14]. Among these factors is membrane surface potential, which is determined by the ionized polar groups of phospholipids and proteins at the membrane surface [15, 16]. The net surface charge in most biological membranes is negative [17–19]. Surface charge of biological and artificial membranes affects membrane permeability to ions and metabolites, as well as the activity of membrane enzymes [20–27].

Several factors modulate that magnitude of the membrane potential:

1. Ionic strength. Inorganic and organic cationic solutes partially screen negative charges on membrane surfaces, which decreases the magnitude of the surface potential in proportion to overall ionic strength. Since the contribution of individual ions to ionic strength is proportional to the square of their charge, divalent cations such as Mg^{2+}

Abbreviations: CsA, cyclosporin A; CTAB, cetyltrimethylammonium bromide; LUV, large unilamellar vesicles; MPT, mitochondrial permeability transition; Pal, palmitic acid; PC, phosphatidylcholine; SDS, sodium dodecylsulfate; SRB, sulforhodamine B

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exert a greater effect on membrane potential than monovalent cations like K^+ and Cl^- [15, 28].
2. pH. Increasing pH promotes the anionic forms of membrane lipids and proteins, which increases the magnitude of the negative surface potential and in turn influences membrane processes [19].
3. Insertion of charged amphiphiles into the membrane bilayer. Charged amphiphilic molecules, for example, cetyltrimethylammonium bromide (CTAB; cationic detergent) and sodium dodecylsulfate (SDS; anionic detergent), insert into the bilayer to increase the density of membrane positive and negative charges, respectively, with a concomitant decrease and increase of the negative surface potential [27, 29].

The objective of the present work was to examine the effect of modulators of surface membrane potential on palmitate/Ca^{2+}-induced permeabilization of liposomal and mitochondrial membranes. We show: 1) The amplitude and rate of the palmitate/Ca^{2+}-induced CsA-insensitive swelling of rat liver and heart mitochondria were lower in high ionic strength than low ionic strength medium. 2) High ionic strength also inhibited palmitate/Ca^{2+}-induced permeabilization of liposomes. 3) The anionic detergent SDS and the negatively charged phospholipid cardiolipin increased the magnitude of the negative ζ-potential of liposomes, whereas the cationic detergent CTAB reversed the ζ-potential of liposomes from negative to positive; 4) CTAB suppressed opening of palmitate/Ca^{2+}-induced pores in mitochondria and liposomes, whereas SDS and cardiolipin augmented the pore formation.

2. Materials and methods

2.1. Materials

Medium components, inorganic chemicals, fatty acids, sulforhodamine B (SRB), CsA, CTAB, SDS, and phosphatidylcholine (PC) were purchased from Sigma-Aldrich (USA). Cardiolipin was purchased from Avanti Polar Lipids (USA).

2.2. Isolation of rat mitochondria

Mitochondria were isolated from livers and hearts of Wistar rats (220–250 g) by differential centrifugation, as described [9]. The homogenization buffer contained 210 mM mannitol, 70 mM sucrose, 1 mM EDTA, and 10 mM Hepes/KOH buffer, pH 7.4. Subsequent centrifugations were performed in the same buffer, except that 100 μM EGTA replaced EDTA. Final suspensions contained 90–100 (liver) and 30–50 (heart) mg of mitochondrial protein/ml, as determined by the Lowry method [30].

2.3. Mitochondrial swelling

Swelling of mitochondria (0.4 mg/ml) was measured as a decrease of A_{540} in a stirred cuvette at room temperature (~22 °C) using an USB-2000 spectroscopy fiber-optic system (Ocean Optics, USA). The incubation medium was 210 mM mannitol, 70 mM sucrose, 5 mM succinate, 5 μM EGTA, 1 μM rotenone, 1 μM CsA, and 10 mM Hepes/KOH buffer, pH 7.4, or 120 mM KCl, 5 μM EGTA, 1 μM rotenone, 1 μM CsA, and 10 mM Tris/HCl buffer, pH 7.4.

2.4. Preparation of large unilamellar liposomes

Large unilamellar vesicles (LUV) were prepared by an extrusion technique, as described [11]. Dry egg PC (0.75 mg) was hydrated for several hours with periodic vortexing in 0.75 ml of buffer containing 40 mM SRB, 10 mM Tris-HCl (pH 8.5), and 50 μM EGTA. After five cycles of freezing/thawing at −20/+30 °C, the suspension was pressed 11 times through a 0.1 μm polycarbon membrane using an Avanti microextruder (Avanti Polar Lipids, USA). All operations except freezing/thawing were carried out at room temperature. After extrusion, liposomes were applied onto a Sephadex G-50 column to remove external SRB. The buffer for gel filtration was 40 mM KCl, 50 μM EGTA, and 10 mM Tris-HCl, pH 8.5. SRB was self-quenched inside LUV. Accordingly, release of SRB was estimated from the increase of SRB fluorescence (unquenching) in buffer containing 40 mM KCl, 50 μM EGTA, and 10 mM Tris-HCl (pH 8.5) as described [11]. Fluorescence was measured using a USB-2000 spectroscopy system at excitation and emission wavelengths of 565 and 586 nm, respectively.

2.5. ζ-potential

The ζ-potential is electrical potential at the hydrodynamic plane of shear, which is generally proportional to the surface potential although slightly smaller in magnitude. ζ-potential like surface potential is related to the membrane surface charge density. ζ-potential was determined by the Helmholtz–Smoluchowski relationship [31] from the electrophoretic mobility of LUV suspensions (0.05 mM total lipid) measured with a Zetasizer Nano-ZS (Malvern Instruments, Malvern, UK) at 25 °C in buffer containing 40 mM KCl, 50 μM EGTA, and 10 mM Tris-HCl, pH 8.5.

2.6. Statistical analysis

The data were analyzed using the GraphPad Prism 5 and Excel software and were presented as means ± SEM of 3–7 experiments. Statistical differences between means were determined by a two-tailed t test using $p < 0.05$ as the criterion of significance.

3. Results

3.1. Effects of ion composition and pH on cyclosporine A-insensitive permeabilization of mitochondria induced by palmitic acid and Ca^{2+}

To assess the effects of ion composition and pH on palmitate/Ca^{2+}-induced permeabilization, absorbance of liver mitochondria in the presence of CsA was measured after addition of 15 μM palmitic acid and then 30 μM Ca^{2+}. In sucrose/mannitol medium, the amplitude and rate of the mitochondrial swelling were substantially greater than in KCl medium (Fig. 1A, compare trace 1 to trace 2, and 1B). Moreover, after high-amplitude swelling was completed, liver mitochondria showed a tendency to shrink in KCl medium, as evidenced by slowly increasing absorbance (Fig. 1A, trace 2). Palmitate/Ca^{2+} also induced swelling of heart mitochondria in KCl medium that was maximal after about 2 min (Fig. 1C). After maximal swelling, heart mitochondria completely restored their volume over the next several minutes.

The rate of mitochondrial swelling induced by 15 μM palmitic acid and 30 μM Ca^{2+} was also evaluated as a function of pH in liver mitochondria. Rates of swelling increased as pH increased from 7.0 to 7.8 (Fig. 2). At 8.0 and above, palmitic acid alone induced mitochondrial swelling, consistent with previous reports [32, 33].

3.2. Ionic strength and permeabilization of liposomes by palmitic acid and Ca^{2+}

In buffer containing 80 mM sucrose, successive additions of 15 μM palmitic acid and then 1 mM $CaCl_2$ led to release of 64% of SRB from liposomes (Fig. 3). SRB release decreased to 44% in buffer containing 40 mM KCl, which was essentially the same as SRB release when KCl was replaced by LiCl, NaCl, RbCl, and CsCl (Fig. 3). As KCl increased from 0 to 40 mM and sucrose decreased isotonically from 80 to 0 mM, SRB release decreased. Only a slight decrease was observed as KCl increased from 0 to 5 mM, but as KCl increased from 10 to 40 mM, SRB release became a nearly inverse linear function of KCl concentration (Fig. 4). Thus, the ionic strength of the buffer affects not only palmitate/

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