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Methyl-beta-cyclodextrin induces mitochondrial cholesterol depletion and alters the mitochondrial structure and bioenergetics

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This paper is dedicated to our mentor and friend Prof. Jerzy Popinigis, who has inspired us to engage in the mitochondriology

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ABSTRACT

There is growing evidence of mitochondrial membrane raft-like microdomains that are involved in the apoptotic pathway. The aim of this study was to investigate the effect of methyl-beta-cyclodextrin (MβCD), being a well-known lipid microdomain disrupting agent and cholesterol chelator, on the structure and bioenergetics of rat liver mitochondria (RLM). We observed that MβCD decreases the function of RLM, induces changes in the mitochondrial configuration state and decreases the calcium chloride-induced swelling. These data suggest that disruption of mitochondrial raft-like microdomains by cholesterol efflux on one hand impairs mitochondrial bioenergetics, but on the other hand it protects the mitochondria from swelling.

1. Introduction

There are at least four main membrane lipid microdomains located within the plasma membrane, mitochondrial membranes, golgi apparatus and endoplasmic reticulum [1–4]. The plasma membrane "lipid rafts" consist of cholesterol and sphingolipids [1].

There is also growing evidence suggesting the existence of mitochondrial raft-like microdomains [4,5], which are involved in the signaling pathways controlling the apoptotic process [6,7].

Even if the cholesterol content in mitochondria is lower than in other organelles, its appropriate level for mitochondrial raft-like microdomains seems to be crucial [4,5]. In rat liver mitochondria (RLM), cholesterol is mainly distributed within the areas of outer membrane (OM), involved in creation of mitochondria contact sites [8], joining mitochondrial membranes, where voltage-dependent anion channel (VDAC) is localized on the inside or outside of cholesterol-containing lipid raft-like areas [9].

Mitochondrial contact sites are also associated with the mitochondrial permeability transition pore (mPTP) being a multiprotein complex, comprising, among others, adenine nucleotide translocator (ANT), VDAC, peripheral benzodiazepine receptor, creatine kinase, hexokinase II, cyclophiline D, and BAX/Bcl-2-like proteins. Activation of mPTP is associated with mitochondrial

Abbreviations: ANT, adenine nucleotide translocator; COX, cytochrome *c* oxidase; CSA, cyclosporin A; DPM, disintegrations per minute; GD3, disialoganglioside; MAM, mitochondria-associated membranes; MβCD, methyl-beta-cyclodextrin; mDRMs, mitochondrial detergent-resistant membranes; mPTP, mitochondrial permeability transition pore; OM, outer membrane; RCI, respiratory control index; RLM, rat liver mitochondria; t-Bid, pro-apoptotic protein; VDAC, voltage-dependent anion channel

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depolarization, uncoupling of oxidative phosphorylation, swelling of mitochondria and release of death-promoting factors like cytochrome *c* [10]. There are many factors implicated in mPTP regulation, i.e., oxidative stress [11], mitochondrial GSH depletion, Ca^{2+} and Pi, lowering of extramitochondrial ATP [12], and ADP level [13], collapse of mitochondrial membrane potential, and decrease of matrix pH [14,15]. In addition, it has been shown that mitochondrial raft-like microdomain specific lipids play a key role in the regulation of mPTP [5]. Furthermore, it has been proposed that lipid microdomains, enriched in cholesterol and ceramide, could coexist as structural elements with some mPTP-forming proteins, and with members of the Bcl-2 family [16]. In the reperfused rat heart model, the release of cytochrome *c* from mitochondria correlates with Bax insertion into the mitochondrial detergent-resistant membranes (mDRMs), which are abundant in VDAC, ANT, cholesterol and ceramide [16].

Methyl-beta-cyclodextrin (MβCD) belongs to the β-cyclodextrin family, being capable of removing cholesterol from the plasma membrane [17]. The methylated form is more efficient cholesterol chelator than non-methylated β-cyclodextrin [18]. MβCD molecule has an internal hydrophobic cavity that can accommodate normally insoluble compounds such as cholesterol. The surface hydrophilicity of cyclodextrin-cholesterol complex accounts for its solubility in aqueous solutions [19]. Garofalo et al. [4] reported that MβCD-induced disruption of raft-like microdomains in isolated mitochondria from human lymphoblastoid CEM cells prevented mitochondria depolarization and cytochrome *c* release after treatment with disialoganglioside (GD3) or t-Bid, pro-apoptotic protein. Also in another study, an inhibitory effect of MβCD on rBAX-induced opening of mPTP was observed [5]. MβCD lowered cholesterol and ganglioside content in the mitochondria of control rat kidney cortex. Similar resistance to mPTP opening induced by rBAX, was observed in kidney mitochondria from hypothyroid rats that were characterized by lower cholesterol and ganglioside content [5]. Overall, these data suggest that lipid composition of mitochondrial membrane raft-like microdomains may play an important role in the regulation of early stages of apoptosis.

Therefore, we hypothesized that the disruption of mitochondrial raft-like microdomains by cholesterol removal could affect the mitochondrial structure and energetic metabolism.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma (St. Louis, MO, USA), except for BSA (Merck, Damstadt, Germany).

2.2. Animals

All experiments were approved by the Local Ethical Committee at the Medical University in Gdansk (Consent No. 13/2007).

Wistar rats weighing 250–300 g were housed in an environmentally controlled room (23 ± 1 °C, 12 h/12 h light–dark cycle), and received standard rat chow and water ad libitum.

2.3. Isolation of RLM

Mitochondria were isolated as described earlier [20]. The quality of mitochondrial preparation was checked by Western-blotting analysis. Further purification of crude mitochondria, and isolation of mitochondria-associated membranes (MAM) fraction and pure mitochondria were performed as previously described [21]. Mitochondrial and MAM lysates (40 μg of protein/lane) were separated by SDS–PAGE on 10% gel and transferred onto PVDF membrane (BioRad). Membranes were blocked using 2% non-fat milk in TBS buffer with 0.01% Tween-20 for 1 h. The purity of mitochondrial and MAM fractions was checked using specific antibodies against ADP/ATP carrier (ANT), plasma membrane Ca^{2+}-ATPase (PMCA) and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) followed by incubation with secondary HRP-conjugated antibodies. During the experiment, mitochondria were kept in the form of suspension containing 30–50 mg of protein per 1 ml. The protein content was estimated according to the method of Lowry et al. [22].

2.4. Cholesterol estimation

Isolated RLM (1 mg of protein/ml) were incubated with control respiration buffer or with the buffer containing 2% or 4% MβCD. After 5 min of incubation, samples were centrifuged at 10 000 g for 10 min, and mitochondrial cholesterol was determined as previously described [23].

2.5. Mitochondrial respiratory activity assay

Mitochondrial oxygen uptake was measured at 25 and 37 °C with the Clark oxygen electrode using Gilson Polarograph. The respiration medium contained 170 mM sucrose, 15 mM KCl, 5 mM potassium phosphate, 0.1 mM EDTA, 0.1% BSA, and 5 mM succinate (pH 7.4) was used as an oxidizable substrate. Mitochondria (1 mg of protein/ml) were incubated for 2.5 min in the medium without (control) or in the presence of 2% or 4% of MβCD. Respiratory control index (RCI) and ADP/O were measured and calculated from oxygen electrode traces as previously described [24].

2.6. Mitochondrial enzyme activities measurement

The activity of cytochrome *c* oxidase (COX) was measured according to [25] and the activity of rotenone-insensitive NADH cytochrome *c* reductase was assessed according to [26]. Enzymatic activities measured in mitochondrial pellet (1 mg of protein/ml) obtained after 5 min incubation with respiration buffer were expressed as 100%. The activity of the enzymes assessed in other cases were compared to the control and expressed as percentage of control activity.

2.7. Swelling of the RLM

The measurement of RLM swelling was performed spectrophotometrically as previously described [27]. $CaCl_2$ (100 μM) was used as mPTP opening inducer and cyclosporin A (CSA, 1 μM) as mPTP opening inhibitor. 1 mg of RLM was added to the buffer supplemented with 1 mM succinate and 1 μM rotenone in the presence or absence of 2% or 4% of MβCD.

2.8. EM analyses

Mitochondria (1 mg of protein) were incubated for 5 min with control buffer or buffer supplemented with 2% or 4% of MβCD, or 0.25% digitonin, then fixed and processed for electron microscopy as previously described [28].

2.9. Data analysis

Statistical analysis was performed using a dedicated software package (Statistica v. 7.0, Stat Soft Inc.). Results are expressed as mean ± standard error of the mean (S.E.M.). Differences between means were tested using one-way ANOVA test. The statistical significance was established at $P < 0.05$.

3. Results

3.1. The effect of MβCD on mitochondrial cholesterol content

First, we checked the influence of 2% or 4% MβCD on cholesterol status in RLM (Fig. 1). The mean level of total pool of cholesterol in control RLM was 37.5 ± 3.8 disintegrations per minute (DPM) per mg of mitochondrial protein. Both 2% and 4% MβCD significantly decreased mitochondrial cholesterol to 18.4 ± 4.2 and 10.8 ± 2.9, respectively.

3.2. MβCD decreased activity of rotenone-insensitive NADH cytochrome c reductase

Next, we measured COX and rotenone-insensitive NADH cytochrome *c* reductase activities in order to observe the effect of mitochondrial cholesterol removal on enzymes localized within inner and outer mitochondrial membranes, respectively. As shown in Fig. 2, COX activity was insignificantly lower after MβCD treatment, while the activity of rotenone-insensitive NADH cytochrome *c* reductase decreased by 17% or 35% at 2% or 4% MβCD, respectively.

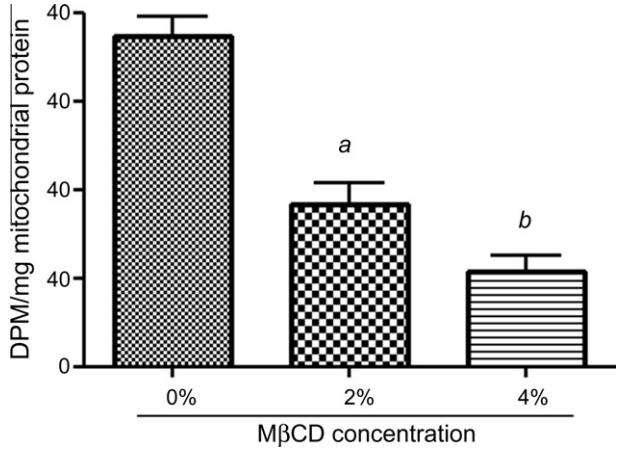

Fig. 1. MβCD induced cholesterol depletion in RLM. RLM were treated with 0%, 2% or 4% MβCD for 5 min and cholesterol content was evaluated in the mitochondrial pellets. The amount of cholesterol in the mitochondrial pellet was measured using radioactive assay, was expressed as disintegrations per minute (DPM) per 1 mg of mitochondrial protein. All data are means ± S.E.M. of at least six independent experiments. $^{a,b}P < 0.001$ is significantly different as compared with 0%.

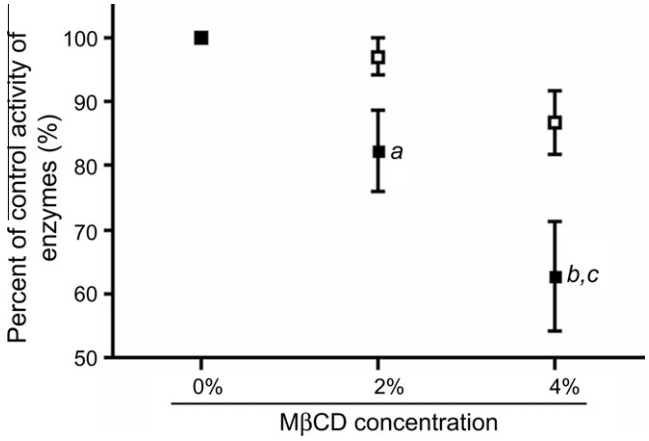

Fig. 2. MβCD-induced mitochondrial cholesterol depletion leads to diminished rotenone-insensitive NADH cytochrome *c* reductase activity. The enzyme activities of marker mitochondrial membranes: OM – rotenone-insensitive NADH cytochrome *c* reductase and IM – cytochrome *c* oxidase, were measured in mitochondrial pellets obtained after incubation with control respiratory buffer 0% (without MβCD), 2% or 4% MβCD. Enzyme activities are expressed as percentage of the relevant control activities. Cytochrome *c* oxidase (□) and rotenone-insensitive NADH cytochrome *c* reductase (■) activities: ^{a,b} significantly differed as compared with control buffer ($P < 0.01$ and $P < 0.001$, respectively); ^{c} was significantly different as compared with 2% MβCD ($P < 0.01$). All data are presented as means ± SEM of at least six independent experiments.

3.3. The effect of MβCD on mitochondrial bioenergetics

One of the main characteristics of mitochondrial function is RCI defined as a ratio of oxygen consumption rate in the presence of ADP to that one observed in the absence of ADP. At 25 °C, 2% MβCD insignificantly lowered RCI from 6.4 ± 0.8 to 5.5 ± 0.8, while 4% MβCD caused RCI decrease to 1.3 ± 0.03 (with $P < 0.001$).

A more evident effect of MβCD on RCI was observed at 37 °C. After incubation of RLM with 2% of MβCD, RCI was significantly decreased from 4.5 ± 0.9 to 2.5 ± 0.3 (with $P < 0.01$). In the presence of 4% of MβCD the rate of oxygen consumption was not stimulated by ADP.

Similarly to RCI, at 25 °C only 4% MβCD significantly decreased ADP/O ratio (a marker of mitochondrial coupling) from 2.0 ± 0.1 to 1.5 ± 0.1 ($P < 0.001$). However, at 37 °C mitochondria respiration was also insensitive to ADP addition after treatment of 4%MβCD, whereas 2% MβCD decreased ADP/O ratio from 2.0 ± 0.1 to 1.7 ± 0.1 ($P < 0.01$).

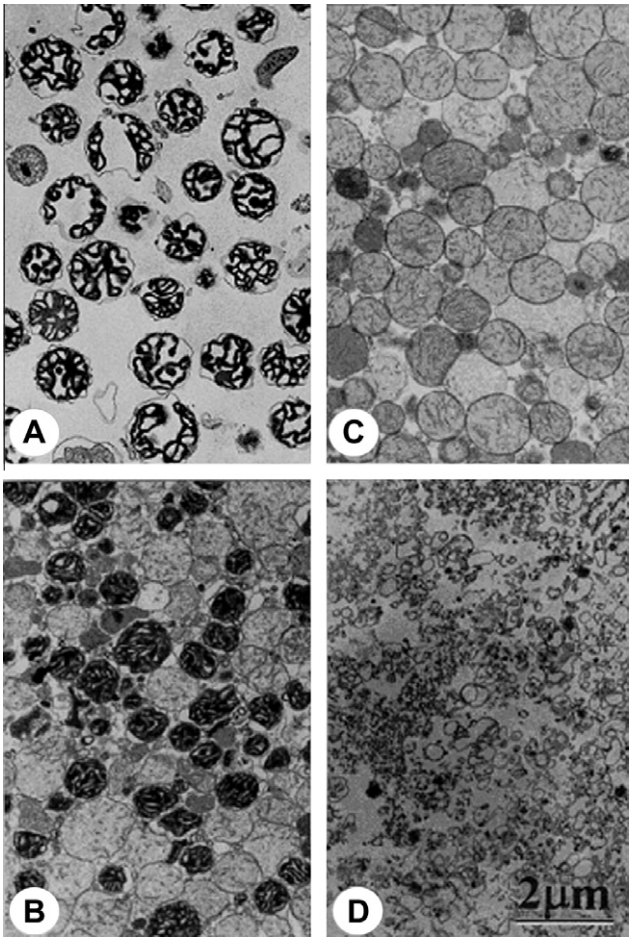

Fig. 3. MβCD-induced mitochondrial configuration change. Electron micrographs of mitochondria from rat liver were isolated and prepared as previously described [28]. Mitochondria were untreated (0% MβCD – Fig. 3A) or were incubated with 2% MβCD (Fig. 3B), 4% MβCD (Fig. 3C), or 0.25% of digitonin (Fig. 3D), as described in Section 2. MβCD-induced configuration state change of RLM from aggregated to orthodox was observed (Fig. 3B and C). Mitochondria were completely disrupted when treated with digitonin (Fig. 3D). Bar, 2 μm.

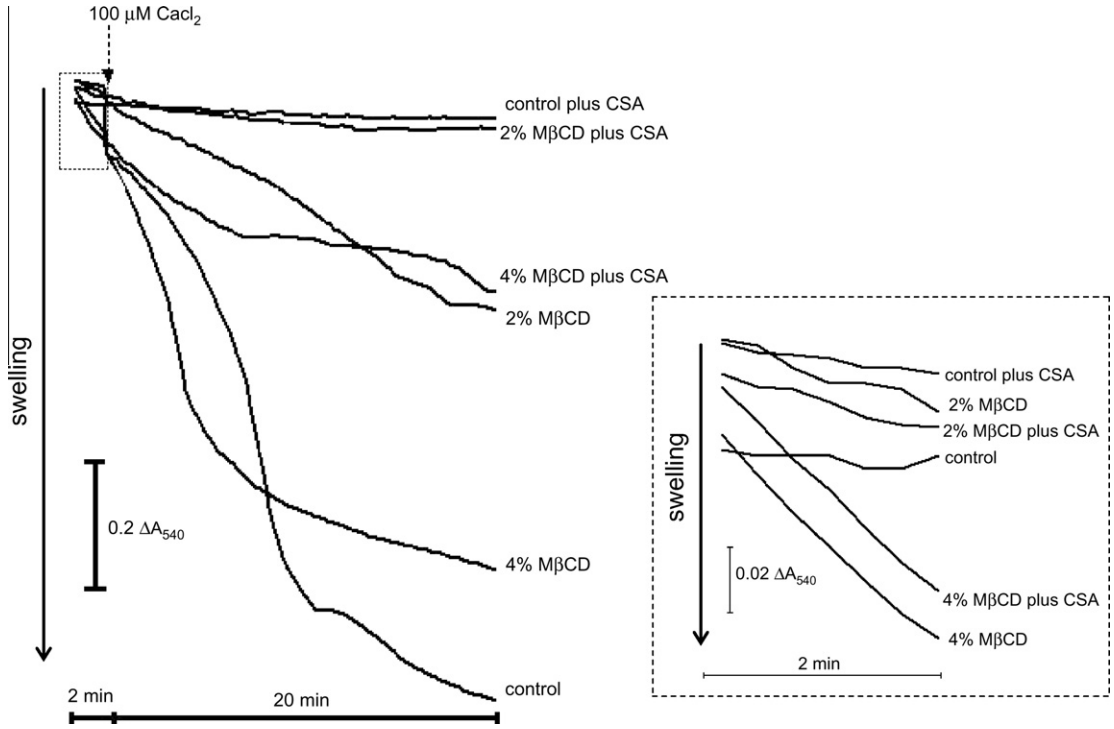

Fig. 4. Dual effect of MβCD on mitochondrial swelling. Mitochondrial swelling induced by MβCD or calcium chloride was assessed spectrophotometrically. Figure on the right (2 min of preincubation with control buffer, 2% or 4% of MβCD) shows the effect of MβCD on mitochondrial swelling. When calcium chloride was not present in the incubation medium, 4% MβCD, induced mitochondrial swelling which was not inhibited by 1 μM CSA. Figure on the left: after 2 min of preincubation (positive control) 100 μM of calcium chloride was added. Inhibitory effect of CSA (added at the beginning of incubation) was observed. MβCD partially reduced $CaCl_2$-induced swelling. Arrow indicates the time when $CaCl_2$ was added. The typical curves of at least three independent experiments are presented.

3.4. MβCD-induced mitochondrial transition from the aggregated to orthodox configuration

Electron microscopic analyses have shown that MβCD treatment of isolated RLM induced conformational changes. As shown in Fig. 3, the control mitochondria were mostly in the aggregated state, while MβCD treatment increased the number of mitochondria in orthodox configuration in a concentration dependent manner. Digitonin, an another compound that is able to disrupt mitochondrial OM, completely disrupted RLM structure (Fig. 3D).

3.5. Mitochondrial swelling induced by calcium chloride is ameliorated by cholesterol depletion

MβCD, during 2 min incubation period preceding $CaCl_2$ addition, induced swelling of RLM at 4%, whereas 2% MβCD had almost no effect (Fig. 4). We did not observe any swelling of control as well as 2% MβCD treated mitochondria for the next 20 min (data not shown).

However, the mitochondrial swelling induced by calcium chloride was partially prevented by cholesterol depletion (Fig. 4). The inhibitory effect of MβCD on RLM swelling was more efficient at 2% as compared to 4% concentration. CSA, an inhibitor of mPTP opening, partially decreased RLM swelling induced by $CaCl_2$ in the presence of 4% MβCD, and completely blocked $CaCl_2$ induced swelling in the presence or absence of 2% MβCD.

4. Discussion

In the present study we demonstrated, that MβCD-induced depletion of mitochondrial cholesterol, being the main component of mitochondrial raft-like microdomains or mDRMs, impaired mitochondrial bioenergetics. Despite of this fact, the MβCD treatment increased mitochondria resistance to calcium chloride-induced swelling.

Appropriate cholesterol content in the mitochondria is important for normal function of the organelle, and cholesterol overaccumulation or decreased cholesterol levels have been observed under physiological and pathological conditions [5,29–31]. For example, reduced cholesterol level was detected in rat hypothyroid kidney cortex mitochondria [5] as well as in rat heart mitochondria isolated after long lasting endurance exercise [30].

A new role of cholesterol as a key element in mitochondrial raft-like microdomain structure has been proposed by group of Zazueta [5]. The changes in mitochondrial cholesterol pool induced by MβCD treatment may influence mitochondrial contact site components. Mitochondrial contact sites and further mPTP, are implicated in mitochondrial apoptotic pathway [12]. Therefore, we hypothesized that disruption of mitochondrial raft-like microdomains by MβCD treatment can prevent the changes observed in mitochondria during an early stage of apoptosis like impairment of mitochondrial bioenergetics and swelling.

In addition, we observed that MβCD was able to induce the change of RLM configuration state from aggregated to orthodox. According to Ardail et al. [8] the majority of mitochondrial cholesterol is located non-homogenously within dynamic structures called contact sites, which are implicated in the physiological control of mitochondrial ultrastructure, e.g., orthodox vs. aggregated transition, as well as mitochondrial bioenergetics. Our results showed that cholesterol depletion shifts mitochondrial configuration state from the aggregated to orthodox one. In addition, depletion of mitochondrial cholesterol, as observed in hypothyroid mitochondria, may influence the rate of ATP synthesis and decrease the efficiency of oxidative phosphorylation [32].

MβCD is known to induce membrane cholesterol efflux and ultimately disruption of mitochondrial raft-like microdomains

[4,16], and thus affecting mitochondrial contact sites. Marzulli et al. [33] observed that external NADH oxidation depends on a number of contact sites. The enzyme responsible for this oxidation is rotenone-insensitive NADH cytochrome *c* reductase. We have shown that MβCD significantly reduced the activity of the enzyme, which indeed suggests that this reductase is functionally connected with mitochondrial raft-like microdomains. Moreover, different effects of MβCD on COX and rotenone-insensitive NADH cytochrome *c* reductase activities can be explained by heterogenity of cholesterol distribution in RLM. As RLM cholesterol is localized mainly within the OM, we suspected that MβCD influences more mitochondrial outer rather than inner membrane enzyme activities. The results presented on Fig. 2 confirm our hypothesis.

When the swelling of mitochondria was stimulated by calcium chloride, MβCD partially inhibited this process. These data are in agreement with previously reported studies, showing that the disruption of mitochondrial lipid microdomains by MβCD, is associated with inhibition of mPTP and early signs of apoptosis [4,5].

In addition to that, it has been already shown that mitochondria from hepatoma cells exhibit alleviated mitochondrial cholesterol content correlating with malignancy [29]. Altogether, the data published so far and our own results indicate that pharmacological modulation of mitochondrial cholesterol level seems to be of potential clinical importance.

In conclusion, our data suggest that MβCD-induced disruption of mitochondrial raft-like microdomains impairs the bioenergetics of RLM stimulating changes in mitochondrial configuration state, and in the same time partially inhibits calcium chloride-induced mitochondrial swelling.

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References

[1] Pike, L.J. (2004) Lipid rafts: heterogeneity on the high seas. Biochem. J. 378, 281–292.
[2] Ardail, D., Popa, I., Bodennec, J., Louisot, P., Schmitt, D. and Portoukalian, J. (2003) The mitochondria-associated endoplasmic-reticulum subcompartment (MAM fraction) of rat liver contains highly active sphingolipid-specific glycosyltransferases. Biochem. J. 371, 1013–1019.
[3] Morales, A., Colell, A., Mari, M., Garcia-Ruiz, C. and Fernandez-Checa, J.C. (2004) Glycosphingolipids and mitochondria: role in apoptosis and disease. Glycoconj. J. 20, 579–588.
[4] Garofalo, T. et al. (2005) Lipid microdomains contribute to apoptosis-associated modifications of mitochondria in T cells. Cell Death Differ. 12, 1378–1389.
[5] Martinez-Abundis, E., Garcia, N., Correa, F., Franco, M. and Zazueta, C. (2007) Changes in specific lipids regulate BAX-induced mitochondrial permeability transition. FEBS J. 274, 6500–6510.
[6] Garofalo, T. et al. (2007) Do mitochondria act as "cargo boats" in the journey of GD3 to the nucleus during apoptosis? FEBS Lett. 581, 3899–3903.
[7] Malorni, W., Giammarioli, A.M., Garofalo, T. and Sorice, M. (2007) Dynamics of lipid raft components during lymphocyte apoptosis: the paradigmatic role of GD3. Apoptosis 12, 941–949.
[8] Ardail, D., Privat, J.P., Egret-Charlier, M., Levrat, C., Lerme, F. and Louisot, P. (1990) Mitochondrial contact sites. Lipid composition and dynamics. J. Biol. Chem. 265, 18797–18802.
[9] Grimm, S. and Brdiczka, D. (2007) The permeability transition pore in cell death. Apoptosis 12, 841–855.
[10] Bernardi, P., Scorrano, L., Colonna, R., Petronilli, V. and Di Lisa, F. (1999) Mitochondria and cell death. Mechanistic aspects and methodological issues. Eur. J. Biochem. 264, 687–701.
[11] Skulachev, V.P. (2000) Mitochondria in the programmed death phenomena; a principle of biology: "it is better to die than to be wrong". IUBMB Life 49, 365–373.
[12] Crompton, M., Barksby, E., Johnson, N. and Capano, M. (2002) Mitochondrial intermembrane junctional complexes and their involvement in cell death. Biochimie 84, 143–152.
[13] Haworth, R.A. and Hunter, D.R. (1980) Allosteric inhibition of the Ca2+-activated hydrophilic channel of the mitochondrial inner membrane by nucleotides. J. Membr. Biol. 54, 231–236.
[14] Bernardi, P. (1992) Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization. J. Biol. Chem. 267, 8834–8839.
[15] Sugrue, M.M. and Tatton, W.G. (2001) Mitochondrial membrane potential in aging cells. Biol. Signals Recept. 10, 176–188.
[16] Martinez-Abundis, E., Correa, F., Pavon, N. and Zazueta, C. (2009) Bax distribution into mitochondrial detergent-resistant microdomains is related to ceramide and cholesterol content in postischemic hearts. FEBS J. 276, 5579–5588.
[17] Ohtani, Y., Irie, T., Uekama, K., Fukunaga, K. and Pitha, J. (1989) Differential effects of alpha-, beta- and gamma-cyclodextrins on human erythrocytes. Eur. J. Biochem. 186, 17–22.
[18] Klein, U., Gimpl, G. and Fahrenholz, F. (1995) Alteration of the myometrial plasma membrane cholesterol content with beta-cyclodextrin modulates the binding affinity of the oxytocin receptor. Biochemistry 34, 13784–13793.
[19] Pitha, J., Irie, T., Sklar, P.B. and Nye, J.S. (1988) Drug solubilizers to aid pharmacologists: amorphous cyclodextrin derivatives. Life Sci. 43, 493–502.
[20] Adachi, K., Wakabayashi, T. and Popinigis, J. (1991) Effects of alkyl alcohols and related chemicals on rat liver structure and function. II. Some biochemical properties of ethanol-, propanol- and butanol-treated rat liver mitochondria. Acta Pathol. Jpn. 41, 414–427.
[21] Wieckowski, M.R., Giorgi, C., Lebiedzinska, M., Duszynski, J. and Pinton, P. (2009) Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells. Nat. Protoc. 4, 1582–1590.
[22] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
[23] Chmielewski, M., Nieweglowski, T., Swierczynski, J., Rutkowski, B. and Boguslawski, W. (2001) Diurnal rhythm of cholesterol biosynthesis in experimental chronic renal failure. Mol. Cell. Biochem. 228, 33–37.
[24] Estabrook, R.W. (1967) Mitochondrial respiratory control and the polarographic measurements of ADP:O ratio in: Methods in Enzymology (Estabrook, R.W. and Pullman, M.E., Eds.), pp. 41–47, Academic Press, NY.
[25] Wharton, D.C. and Tzagoloff, A. (1967) Cytochrome oxidase from beef heart mitochondria in: Methods in Enzymology (Estabrook, R.W. and Pullman, M.E., Eds.), pp. 245–250, Academic Press, NY.
[26] Hatefi, Y. and Rieske, J.S. (1967) Preparation and Properties of DPNH-Cytochrome *c* Reductase (Complex I-III of the Respiratory Chain) in: Methods in Enzymology (Estabrook, R.W. and Pullman, M.E., Eds.), pp. 225–231, Academic Press, NY.
[27] Crouser, E.D., Gadd, M.E., Julian, M.W., Huff, J.E., Broekemeier, K.M., Robbins, K.A. and Pfeiffer, D.R. (2003) Quantitation of cytochrome *c* release from rat liver mitochondria. Anal. Biochem. 317, 67–75.
[28] Wakabayashi, T., Teranishi, M.A., Karbowski, M., Nishizawa, Y., Usukura, J., Kurono, C. and Soji, T. (2000) Functional aspects of megamitochondria isolated from hydrazine- and ethanol-treated rat livers. Pathol. Int. 50, 20–33.
[29] Campbell, A.M., Capuano, A. and Chan, S.H. (2002) A cholesterol-binding and transporting protein from rat liver mitochondria. Biochim. Biophys. Acta 1567, 123–132.
[30] Keatisuwan, W., Kinjo, M. and Koyama, T. (1991) Changes in phospholipid constituents in mitochondrial membranes after long lasting exercise in rat heart. Life Sci. 48, 2173–2181.
[31] Rouslin, W., MacGee, J., Wesselman, A.R., Adams, R.J. and Gupte, S. (1980) Canine myocardial ischemia: increased mitochondrial cholesterol, a marker of mitochondrial membrane injury. J. Mol. Cell. Cardiol. 12, 1475–1482.
[32] Guerrieri, F., Kalous, M., Adorisio, E., Turturro, N., Santoro, G., Drahota, Z. and Cantatore, P. (1998) Hypothyroidism leads to a decreased expression of mitochondrial F0F1-ATP synthase in rat liver. J. Bioenerg. Biomembr. 30, 269–276.
[33] Marzulli, D., La Piana, G., Fransvea, E. and Lofrumento, N.E. (1999) Modulation of cytochrome *c*-mediated extramitochondrial NADH oxidation by contact site density. Biochem. Biophys. Res. Commun. 259, 325–330.