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Extramitochondrial OPA1 and adrenocortical function

László Fülöp [a], Anikó Rajki [b], Dávid Katona [a], Gergö Szanda [a], András Spät [a,*]

[a] Department of Physiology, Faculty of Medicine, Semmelweis University, Hungary
[b] Laboratory of Molecular Physiology, Hungarian Academy of Sciences, POB 259, H-1444 Budapest, Hungary

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ABSTRACT

We have previously described that silencing of the mitochondrial protein OPA1 enhances mitochondrial Ca^{2+} signaling and aldosterone production in H295R adrenocortical cells. Since extramitochondrial OPA1 (emOPA1) was reported to facilitate cAMP-induced lipolysis, we hypothesized that emOPA1, via the enhanced hydrolysis of cholesterol esters, augments aldosterone production in H295R cells. A few OPA1 immunopositive spots were detected in ~40% of the cells. In cell fractionation studies OPA1/COX IV (mitochondrial marker) ratio in the post-mitochondrial fractions was an order of magnitude higher than that in the mitochondrial fraction. The ratio of long to short OPA1 isoforms was lower in post-mitochondrial than in mitochondrial fractions. Knockdown of OPA1 failed to reduce db-cAMP-induced phosphorylation of hormone-sensitive lipase (HSL), Ca^{2+} signaling and aldosterone secretion. In conclusion, OPA1 could be detected in the post-mitochondrial fractions, nevertheless, OPA1 did not interfere with the cAMP – PKA – HSL mediated activation of aldosterone secretion.

1. Introduction

The precursor of corticosteroids is cholesterol. Cholesterol may be synthetized within the endoplasmic reticulum or taken up from plasma lipoproteins. Cholesterol esters, taken up by endocytosis of receptor-bound LDL particles, are hydrolyzed in the endoplasmic reticulum. More important for steroid biosynthesis is HDL-transported esterified cholesterol, taken up by scavenger receptor B1 (Rone et al., 2009) and hydrolyzed by cholesterol esterase (Rodriguez et al., 1999). The esterase was recently found to be identical with the HSL of lipocytes (Kraemer et al., 2004). Following reesterification cholesterol accumulates in special, phospholipid layer bounded droplets. Rapid increase of cortisol secretion during stress or increased aldosterone secretion during acute fluid loss requires the rapid mobilization of cholesterol stored in these lipid droplets (Hattangady et al., 2011; Vinson et al., 1992). Deesterification is performed again by HSL (Kraemer et al., 2004).

The dual action of HSL is under hormonal control. ACTH, through cAMP-PKA, phosphorylates and thus activates the enzyme (Hirsch and Rosen, 1984; Holm et al., 2000; Kraemer et al., 2004; Trzeciak and Boyd, 1974) and also induces its expression (Granneman and Moore, 2008; Holysz et al., 2011). Calcium ion, the second messenger of angiotensin II, acts via CaMKII to activate (Cherradi et al., 1998) and through p42/p44 mitogen-activated protein kinase (Cherradi et al., 2003) to increase the expression of HSL.

Transfer of the released cholesterol to the side-chain cleaving enzyme cytochrome $P450_{scc}$ (CYP11A1), located in the IMM, is carried out by a complex of cytosolic and mitochondrial proteins (Rone et al., 2009). At least two components of this complex, StAR and the translocator protein TSPO (previously named as peripheral benzodiazepine receptor) are phosphorylated and induced by PKA (Dyson et al., 2008; Fleury et al., 2004; Manna et al., 2002; Midzak et al., 2011). Although Ca^{2+} - mobilizing agonists (through Ca^{2+} and protein kinase C) were also reported to phosphorylate StAR (Betancourt-Calle et al., 2001; Cherradi et al., 1998) their major effect is the induction of StAR expression (Clark et al., 1995; Lucki et al., 2012; Martin et al., 2008).

In adipocytes activated PKA phosphorylates and brings about translocation of the cytosolic HSL to the membrane fraction (Hirsch and Rosen, 1984). This translocation requires the 62-kDa protein perilipin 1 (Plin 1) located on the surface of lipid droplets (Greenberg et al., 1991; Miyoshi et al., 2006). It is assumed that non-phosphorylated Plin 1 inhibits the access of phosphorylated HSL to its substrate (Brasaemle et al., 2009; Sztalryd et al., 2003; Tansey

Abbreviations: $[Ca^{2+}]_m$, mitochondrial Ca^{2+} concentration; AKAP, A-kinase anchoring protein; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; COX IV, cytochrome *c* oxidase IV; db-cAMP, dibutiryl-cAMP; emOPA1, extramitochondrial OPA1; HSL, hormone-sensitive lipase; IMM, inner mitochondrial membrane; IMS, mitochondrial intermembrane space; Mfn 1, mitofusin 1; OMM, outer mitochondrial membrane; OPA1, Optic Atrophy 1; PDI, protein disulfide isomerase; PKA, protein kinase A; Plin, perilipin; StAR, Steroidogenic Acute Regulating Protein; TSPO, (mitochondrial) Translocation Protein (previously peripheral benzodiazepine receptor).

* Corresponding author. Tel.: +36 1 4591500; fax: +36 1 2667480.
E-mail address: spat@eok.sote.hu (A. Spät).

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et al., 2001; Zhang et al., 2003). Albeit PKA-mediated phosphorylation of Plin is not required for the translocation, phosphorylation is essential for hormone-induced lipolysis (Miyoshi et al., 2006). It is assumed that phosphorylation of Plin 1 permits the activation of adipocyte triglyceride lipase (Granneman et al., 2011; Subramanian et al., 2004; Yamaguchi et al., 2007) which provides diacylglycerol for further hydrolysis by HSL (Zimmermann et al., 2004).

A recently described factor participating in hormonally-induced lipolysis is Optic Atrophy 1 (OPA1). OPA1 (and its ortholog Mgm1p in yeast) has been known as a dynamin-related mitochondrial GTPase protein. In cooperation with Mitofusin 1 (Mfn 1) OPA1 induces mitochondrial fusion and its mutation is the most common cause of type 1 autosomal dominant optic atrophy (Belenguer and Pellegrini, 2012; Cipolat et al., 2004). Unexpectedly, OPA1 has been detected in lipocytes on the surface of Plin-coated lipid droplets (Pidoux et al., 2011). Immunocytochemical and immunoprecipitation studies showed that PKA binds to the Plin-associated OPA1 and the formation of this complex results in Plin phosphorylation and lipolysis. OPA1 contains an A-kinase binding domain and thus it may function as an AKAP. In fractionation studies the amount of OPA1 in the lipid droplet fraction far exceeded that in the mitochondrial fraction. Based on these observations a significant role has been attributed to OPA1 in the hormonal control of lipolysis, assuming that OPA1 potentiates the phosphorylation of Plin 1 by PKA and thus makes further steps possible (presumably the activation of adipocyte triglyceride lipase) (Greenberg et al., 2011; Pidoux et al., 2011). We are not aware of studies on emOPA1 in cell types other than adipocyte.

In humans OPA1 has eight mRNA isoforms and the expressed proteins can be separated in five bands between 96 and 84 kDa in Western blots. The two higher-molecular mass bands, the so-called long isoforms are mixture of isoforms 1, 2, 4 and 7 whereas the three short isoforms contain proteolytic products of the long ones and also isoforms 3, 5, 6 and 8. The long isoforms are attached to the IMM, the soluble short ones were found in the IMS partly associated to the OMM (Delettre et al., 2000; Lenaers et al., 2009; Liesa et al., 2009). OPA1 controls the diameter of the junction of cristae (Scorrano et al., 2002) and thus modifies the molecular diffusion between the lumen of the cristae and the IMS (Frey et al., 2002) and/or between the boundary (inner) and crista membrane (Sukhorukov and Bereiter-Hahn, 2009). Knockdown of OPA1 in H295R human adrenocortical cells, probably due to the altered diffusion conditions, facilitates the transfer of cytosolic Ca^{2+} signal into the mitochondrial matrix (Fülöp et al., 2011) resulting in enhanced aldosterone production (Spät et al., 2012).

In addition to HSL another factor participating in lipolysis, Plin1a (formerly Plin A) has also been detected in Y-1 murine adrenocortical cells (Servetnick et al., 1995). Other Plin isoforms, predominantly Plin1c (formerly Plin C), could also be detected following incubation with cholesterol (Hsieh et al., 2012). Importantly, Plin1a was phosphorylated in a cAMP-dependent manner (Servetnick et al., 1995). The data showing that identical molecules participate in the control of lipid metabolism in adipocytes and adrenocortical cells prompted us to examine whether OPA1, functioning as an AKAP, is a feasible candidate for regulating cAMP-induced steroid secretion. The verified and hypothetical mechanisms supplying cholesterol to mitochondria are shown in Fig. 1. We presumed that silencing of *OPA1*, if functioning as an AKAP in the extramitochondrial space, would reduce PKA-mediated steroid production to a greater extent than PKA-independent response. Our observations indicate that OPA1 is present in the extramitochondrial compartment in H295R cells but the role of emOPA1 in the control of steroid secretion could not be demonstrated.

2. Materials and methods

2.1. Materials

NIH-H295R cells (ATCC, CRL-2128) were purchased from LGC Standards Gmbh, Wesel, Germany. siRNA and silencing RNA products as well as OPTI-MEM, Lipofectamine 2000, Fluo-4 and Mito-Tracker Deep Red were purchased from Life Technologies (Paisley, UK).

CholEsteryl-BODIPY FL C_{12} (C-3927MP) was from Life Technologies (San Diego, CA, USA), UltroSer G was from Bio Sepra (Cergy-Saint-Christophe, France). 2mt-eGFP (eGFP fused with a doublet of human cytochrome *c* oxidase target sequence) was a gift from Dr. B. Enyedi (Budapest, Hungary). Coat-A-Count RIA kit was purchased from Siemens Health Care Diagnostics (Los Angeles, CA).

Primary antibodies were purchased as follows: anti-OPA1 monoclonal antibody (612606): BD Bioscience (Franklin Lakes,

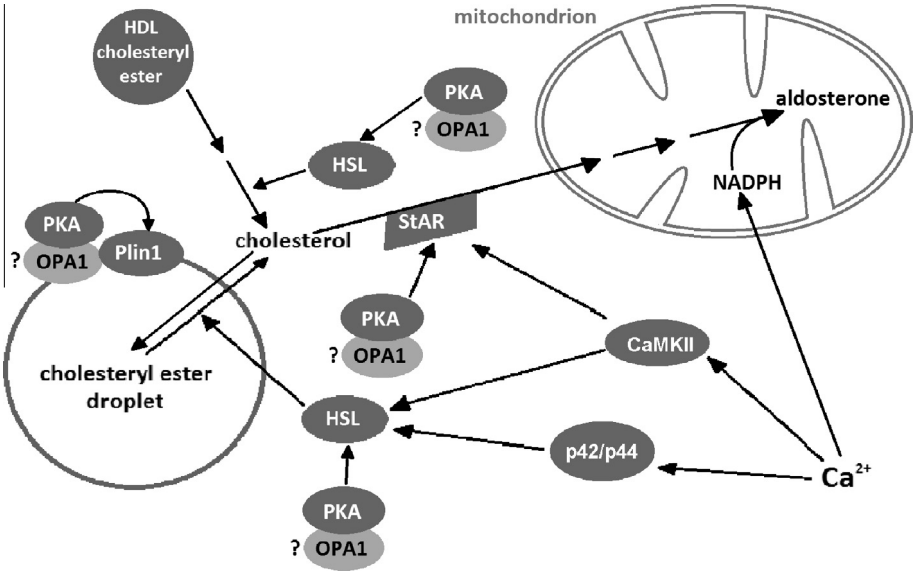

Fig. 1. Cholesterol supply to mitochondria in adrenal glomerulosa cells. The presence and actions of extramitochondrial OPA1 are hypothetical as shown with question marks.