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Review

Control mechanisms of mitochondrial Ca^{2+} uptake – feed-forward modulation of aldosterone secretion

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ABSTRACT

Mitochondrial Ca^{2+} signal activates metabolism by boosting pyridine nucleotide reduction and ATP synthesis or, if Ca^{2+} sequestration is supraphysiological, may even lead to apoptosis. Although the molecular background of mitochondrial Ca^{2+} uptake has recently been elucidated, the regulation of Ca^{2+} handling is still not properly clarified. In human adrenocortical H295R cells we found a regulatory mechanism involving p38 MAPK and *novel-type* PKC isoforms. Upon stimulation with angiotensin II (AII) these kinases are activated typically prior to the release of Ca^{2+} and – most probably by reducing the Ca^{2+} permeation through the outer mitochondrial membrane – attenuate mitochondrial Ca^{2+} uptake in a feed-forward manner. The biologic significance of the kinase-mediated reduction of mitochondrial Ca^{2+} signal is also reflected by the attenuation of AII-mediated aldosterone secretion. As another feed-forward mechanism, we found in HEK-293T and H295R cells that Ca^{2+} signal evoked either by IP_3 or by voltage-gated influx is accompanied by a concomitant cytosolic Mg^{2+} signal. In permeabilized HEK-293T cells Mg^{2+} was found to be a potent inhibitor of mitochondrial Ca^{2+} uptake in the *physiologic* $[Mg^{2+}]$ and $[Ca^{2+}]$ range. Thus, these inhibitory mechanisms may serve not only as protection against mitochondrial Ca^{2+} overload and subsequent apoptosis but also have the potential to substantially alter physiological responses.

Contents

1. Introduction

Mitochondria effectively sequester Ca^{2+} during physiological Ca^{2+} signals (Santo-Domingo and Demaurex, 2010) and thereby substantially modify the Ca^{2+} homeostasis of the cell (Demaurex et al., 2009; Walsh et al., 2009). Furthermore, numerous processes depending on $[Ca^{2+}]_c$ such as exocytosis (Montero et al., 2000; Kaftan et al., 2000), store-operated Ca^{2+} influx (Parekh, 2008) and gene expression (Kim and Usachev, 2009) are also adjusted by mitochondrial Ca^{2+} uptake and subsequent Ca^{2+} efflux. On the

Abbreviations: AII, angiotensin II; $[Ca^{2+}]_c$, cytosolic or (in permeabilized cells) extramitochondrial $[Ca^{2+}]$; $[Ca^{2+}]_m$, mitochondrial $[Ca^{2+}]$; ER, endoplasmic reticulum; FCCP, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; GFP, green fluorescent protein; IP_3, inositol 1,4,5-trisphosphate; $[Mg^{2+}]_c$, cytosolic $[Mg^{2+}]$; nPKC, *novel-type* protein kinase C; OPA1, optic atrophy 1 protein; PDD, 4α-phorbol-didecanoate; PDI, protein disulfide isomerase; PM, plasma membrane; PMA, phorbol myristyl acetate; t-Bid, truncated Bid; TNFα, tumor necrosis factor α; YFP, yellow fluorescent protein; ψ_m, mitochondrial membrane potential.

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other hand, the outcome of net mitochondrial Ca^{2+} uptake, i.e. the elevation of intramitochondrial $[Ca^{2+}]$ ($[Ca^{2+}]_m$), has its own physiological or pathological consequences.

One obvious result of mitochondrial Ca^{2+} sequestration is the enhancement of NADH and NADPH (NAD(P)H) formation. In mitochondrial suspension, three mitochondrial dehydrogenases (pyruvate, isocitrate and 2-oxoglutarate-dehydrogenase) display Ca^{2+}-dependent activity (McCormack, 1985; McCormack et al., 1990) thereby offering a possible regulation of mitochondrial NAD(P)H production by Ca^{2+} signaling. The validity of this assumption in respect of intact cells was demonstrated in adrenal glomerulosa cells (Pralong et al., 1992; Rohács et al., 1997b) and sensory neurons (Duchen, 1992) in which mitochondrial NAD(P)H formation exhibited Ca^{2+} dependent activation. These observations suggested that cytosolic and mitochondrial Ca^{2+} signals are closely linked via mitochondrial Ca^{2+} uptake which was directly demonstrated by Rizzuto et al. (1992) and Hajnóczky et al. (1995). Enhanced reduction of matrix pyridine nucleotides also gives rise to augmented generation of ATP (Jouaville et al., 1999). Thus, mitochondrial Ca^{2+} uptake may adjust energy metabolism to the actual cellular demands.

Mitochondrial Ca^{2+} signal regulates not only energy metabolism but in endocrine cells specific cellular functions have been shown to depend on $[Ca^{2+}]_m$. In adrenal glomerulosa cells aldosterone secretion displays tight correlation with mitochondrial [NAD(P)H] (Pralong et al., 1992) and, importantly, this NAD(P)H is utilized during AII-induced aldosterone production (Rohács et al., 1997a) suggesting that intramitochondrial reduced pyridine nucleotides are not in excess. Since NAD(P)H provides the reducing power for the enzymatic reactions of steroid synthesis these data strongly suggest that intramitochondrial NAD(P)H production and, consequently, $[Ca^{2+}]_m$ may substantially affect hormone production. Indeed, selective buffering of intramitochondrial Ca^{2+} dampens both AII-stimulated aldosterone and glucose-induced insulin secretion (Wiederkehr et al., 2011) showing that mitochondrial Ca^{2+} signal can be a potentiating signal for cell specific responses. It should be also noted that under pathological conditions, when the $[Ca^{2+}]_c$ elevation is supraphysiological, excessive mitochondrial Ca^{2+} load may lead to the opening of the mitochondrial permeability transition pore, the loss of mitochondrial membrane potential, the abrupt oxidation of NAD(P)H and, eventually, to apoptosis (Bernardi and Rasola, 2007).

Ca^{2+} uptake into mitochondria occurs via the recently identified mitochondrial Ca^{2+} uniporter (De Stefani et al., 2011; Baughman et al., 2011). Functionally, the uniporter is an inwardly rectifying Ca^{2+} selective channel in the inner mitochondrial membrane (Kirichok et al., 2004; Michels et al., 2009) and the associated EF-hand protein MICU1 seems to be its Ca^{2+} sensing subunit (Perocchi et al., 2010). Since these key elements of Ca^{2+} sequestration have only recently been identified, important as it may be, the regulation of mitochondrial Ca^{2+} uptake is far from being understood. (As to the regulation of Ca^{2+} transport mechanisms in isolated mitochondria and to the possible involvement of Letm1 in mitochondrial Ca^{2+} handling see Gunter and Sheu (2009) and Jiang et al. (2009), respectively.) However, since mitochondrial Ca^{2+} uptake and $[Ca^{2+}]_m$ have far-reaching effect on energy metabolism, specific cell functions and even cell fate, it can be assumed that fine adjustment of mitochondrial Ca^{2+} uptake could be an efficient tool to regulate these processes and to avoid possibly harmful mitochondrial Ca^{2+} overload.

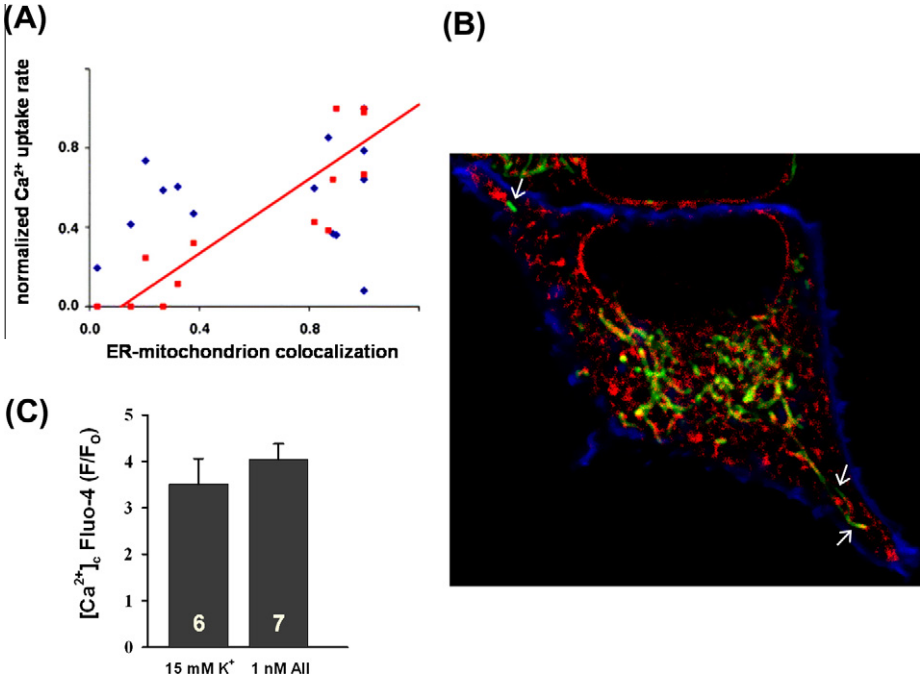

Fig. 1. Location dependent mitochondrial Ca^{2+} uptake in H295R cells. (A) H295R cells expressing ER-targeted GFP were loaded with Rhod-2 ($[Ca^{2+}]_m$) and stimulated with 15 mM K^+ (blue diamonds) and, after $[Ca^{2+}]_m$ has returned to the control level, with 1 nM AII (red squares). Fluorescent changes were monitored with confocal microscopy; each data point represents a small mitochondrial region of interest (ROI). (As found in *z*-stack measurements, GFP fluorescence intensity of ER vesicles inversely correlates with their distance from the optical slice.) The rate of $[Ca^{2+}]_m$ increase and GFP fluorescence were both normalized to the ROI exhibiting the largest GFP fluorescence in the respective cell. The normalized Ca^{2+} uptake rate is shown in the function of GFP fluorescence. For further methodological details see Szanda et al. (2006). Reprinted from Szanda et al. (2006) with permission. (B) H295R cells expressing plasma membrane-targeted YFP (blue; target sequence: palmitoylation sequence of human GAP-43) were loaded with MitoTracker Deep Red (green) and with Endoplasmic Reticulum Tracker Red (red); image acquisition was carried out with confocal microscopy. The majority of mitochondria is found in the perinuclear region. Colocalization of mitochondria (green) with the ER (red) is indicated by yellow color. Mitochondria not overlapping with the ER are typically located in the vicinity of the PM, in the periphery of the cell (arrows). (PM-targeted YFP was kindly provided by Dr. Péter Várnai.) (C) H295R cells were loaded with Fluo-4 ($[Ca^{2+}]_c$) and stimulated with 15 mM K^+ or with 1 nM AII. Fluorescent changes were monitored with confocal microscopy in the periphery of the cells and normalized to the fluorescent value measured before cell stimulation (F_o). Means + SEM of peak Ca^{2+} responses are shown.