Feed-forward control of mitochondrial Ca^{2+} uptake

PhD thesis

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Introduction

Ca^{2+} is one of the most common and versatile second messenger in the eukaryotic cell. Cytoplasmic concentration of Ca^{2+} ($[Ca^{2+}]_c$) is a strictly regulated parameter affecting numerous physiological and pathophysiological processes such as hormone and neurotransmitter secretion, muscle contraction, transcription, apoptosis and necrosis.

It became apparent in the last two decades that mitochondria function not only as Ca^{2+} "buffer" but in fact play an active role in the Ca^{2+} homeostasis of the cell. The role of mitochondria is not confined to the regulation of $[Ca^{2+}]_c$ namely mitochondrial Ca^{2+} signal itself regulates several pivotal cellular functions. Elevated $[Ca^{2+}]$ in the mitochondrial matrix, the so-called mitochondrial Ca^{2+} signal, activates reduction of pyridine-nucleotides and boosts oxidative phosphorylation. Additionally, some cell specific functions such as steroid production or insulin secretion are modulated by mitochondrial matrix free $[Ca^{2+}]$ ($[Ca^{2+}]_m$). On the other hand, $[Ca^{2+}]_m$ exceeding physiological levels may lead to apoptosis or necrosis. Although mitochondrial Ca^{2+} signal is of upmost significance in the aspect of cell functions and cell faith, regulation of mitochondrial Ca^{2+} handling is insufficiently understood.

Mitochondrial Ca^{2+} uptake is mediated by the mitochondrial Ca^{2+} uniporter (MCU), a highly selective inward-rectifying Ca^{2+} channel in the inner mitochondrial membrane. Ca^{2+} uptake into energized mitochondria is driven chiefly by the mitochondrial membrane potential ($\Delta\Psi_m$, ~ 180 mV, inside negative) which is generated by the H^+ extrusion activity of the respiratory chain. Ca^{2+} can leave the matrix through the Na^+/Ca^{2+} or the Ca^{2+}/H^+ antiporters. The capacity of these exchangers is about two orders of magnitude lower than that of the uniporter. Therefore, during cytosolic Ca^{2+}

signal Ca^{2+} uptake soon exceeds Ca^{2+} efflux resulting in a mitochondrial Ca^{2+} response. Mitochondrial Ca^{2+} uptake and the generation of mitochondrial Ca^{2+} signal can be mainly attributed to the MCU.

Mitochondrial Ca^{2+} uptake is a high capacity but low affinity transport. Half-maximal transport velocity of Ca^{2+} is attained (EC_{50}) in the range of 1-200 μM. Strikingly, electrophysiological studies revealed an even higher EC_{50} value (10-20 mM). Since cytosolic Ca^{2+} elevations as low as 300-500 nM are capable of bringing about significant $[Ca^{2+}]_m$ increases, the high EC_{50} of mitochondrial Ca^{2+} uptake gives rise to an apparent contradiction. To resolve this contradiction the so-called high Ca^{2+} microdomain theory was proposed. This idea presumes that in the confined space between the Ca^{2+} source (endoplasmic reticulum or plasma membrane) and the opposing mitochondria local $[Ca^{2+}]$ can reach much higher levers than in the "global" (measurable) cytosol. This narrow space is the high-Ca^{2+} microdomain (HCMD) in which the $[Ca^{2+}]$ is now capable of activating the low affinity MCU.

Formation of HCMDs is widely accepted and is supported by numerous indirect and some recent direct evidences. In fact, HCDM is the dominating theory of mitochondrial Ca^{2+} uptake in intact cells. On the other hand, some data question the exclusivity of HCMD-dependent mitochondrial Ca^{2+} uptake. Slowly emerging cytosolic Ca^{2+} signals in the 200-300 nM concentration-range induce mitochondrial Ca^{2+} uptake in some endocrine and immune cells (adrenal glomerulosa and ovarian granulosa cells, β-cell, T-cell). Moreover, substrate-induced mitochondrial hyperpolarization alone can evoke elevation of $[Ca^{2+}]_m$. Formation of HCMDs under such conditions is highly unlikely (or even impossible) clearly showing that mitochondrial Ca^{2+} uptake does not necessarily require HCMD.

Different cell types may have different toolkits for the regulation of mitochondrial Ca^{2+} uptake. This variability might account for the wide EC_{50}-range of mitochondrial Ca^{2+} uptake reported in the literature. Since the sequence of the MCU is still unknown, factors regulating mitochondrial Ca^{2+} uptake (and thus setting the affinitiy or capacity of the transport) are unknown. ATP and Mg^{2+} were shown to attenuate Ca^{2+} uptake into isolated mitochondria but their effect in the physiological concentration-range has not yet been elucidated. It should also be noted that mitochondrial Ca^{2+} handling has been shown to be affected by the overexpression of some protein kinase C (PKC) isoforms. However, since overexpression does not prove *physiological* significance the role of PKCs needs further examination.

In our experiments we examined the possibility of a HCMD-independent mitochondrial Ca^{2+} uptake in cell types which also exhibit HCMD-dependent Ca^{2+} uptake. If there are both HCMD-dependent and independent Ca^{2+} uptake, what factors define or modulate the affinity of Ca^{2+} uptake? We also investigated the effect of Mg^{2+} on mitochondrial Ca^{2+} uptake in the physiological $[Mg^{2+}]$-range.

The human adrenocortical H295R cell line was used in the majority of our experiments. H295R cell was a reasonable choice to address the aforementioned questions since i) it is an endocrine-derived cell, thus exhibiting mitochondrial Ca^{2+} uptake even in the 10^{-7} M $[Ca^{2+}]_c$-range, ii) cytosolic Ca^{2+} response can be evoked by IP_3-mediated release and also by influx via voltage-dependent or store-operated Ca^{2+} channels, iii) the mitochondrial effect of cytosolic Ca^{2+} signals of different origin can be compared, iv) unlike their primary counterpart H295R cells can be transfected with DNA and siRNA with an acceptable efficiency, v) no laboratory animals are needed if using a cell line. Experiments requiring

highly efficient transfection or high cell density were carried out on HEK-293T or on COS-7 cells.

Aims

We sought to answer the following questions:

1. Is there HCMD-independent mitochondrial Ca^{2+} uptake in cell types which also exhibit HCMD-dependent ion uptake?
2. What factors do regulate or modulate the rate of mitochondrial Ca^{2+} uptake? Do these factors define the HCMD-dependency of mitochondrial Ca^{2+} uptake?
3. Does Mg^{2+} have any regulatory effect on mitochondrial Ca^{2+} uptake in the physiological cytosolic [Mg^{2+}]-range?

Materials and methods

Cell culture and transfection

H295R, HEK-293T and COS-7 cells were cultured at 37 °C (in 5% CO_2) for 3-4 days according to the guidelines of ATCC®. Cell density was 20.000-80.000 cells/coverslip (25 mm diameter) or 100.000-150.000 cells/well (35 mm diameter) in case of fluorescent measurements or immunoblot experiments, respectively. Cells were transfected on day 2 in OPTI-MEM for 4-6 hours using lipid-based transfection reagents. Measurements were conducted on day 3 or 4.

Confocal and microfluorimetric microscopy, luminometry

For confocal measurements the Zeiss LSM510 laser scanning microscope system equipped with 40×/1.3 or 60x/1.6 oil immersion objective (Plan-Neofluar, Zeiss) was used. In microfluorimetric experiments fluorescence was measured using a photomultiplier-based micro-spectrofluorimeter (DeltaRAM, PTI) connected to an inverted microscope (Zeiss) fitted with a 100×/1.30 oil immersion objective (Fluar, Zeiss). Luminescence was measured with a Cairn luminometer.

As a standart extracellular solution Krebs-Ringer medium was used (with the following modifications: 3.6 mM K^+, 1.2 mM Ca^{2+}, 0.5 mM Mg^{2+}, 5 mM Hepes és 2 mM HCO_3^-; pH was 7.4). The loading of fluorescent dyes or pharmacological pre-incubation – if any – were also performed in this solution.

The following cytosol-like solution was used for the perfusion of permeabilized cells (in mM): KCl (117), NaCl (6), KH_2PO_4 (1), $MgCl_2$ (1.13), K-Hepes (10), Na-pyruvate (2), Na-succinate (2), K-ADP (2), K-EGTA (2); pH 7.1. Free concentration of Ca^{2+} and that of Mg^{2+} were

adjusted by the addition of metal chelators according to the calculation of the Chelator software. [Ca^{2+}] and [Mg^{2+}] were checked with a Ca^{2+} selective electrode and/or with fluorescent dyes.

Measurement of $[Ca^{2+}]_c$, $[Ca^{2+}]_m$, $[Mg^{2+}]_c$ and cytosolic [ATP]

$[Ca^{2+}]_c$ was monitored with Fluo-4, Fura Red or with Rhod-2, $[Ca^{2+}]_m$ was measured with Rhod-2, mitochondria-targeted inverse-Pericam (mt-inv-Pericam) or with mitochondria-targeted aequorin. Changes in $[Mg^{2+}]_c$ were detected using Mag-Fluo-4 or Magnesium Green fluorescent dyes. TMRE was used for measuring $\Delta\Psi_m$. Cytosolic [ATP] was monitored by using the luciferine-luciferase assay and luminometry. Fluorescent dyes were loaded into cells in acetoxymethyl-esther form.

Fluo-4, Mag-Fluo-4, Magnesium Green and mt-inv-Pericam were excited at 488 nm, their emission was measured between 500-550 nm. Rhod-2 and TMRE were excited at 543 nm, emitted light was filtered using a 565-nm long-pass filter. Fura Red was excited at 488 nm, its emission was monitored over 650 nm. UV-photolysis was induced with 363 nm laser light.

Experiments were conducted at 37 °C. Cells were perfused with a gravity-driven superfusion system regulated with solenoid valves. Acquired fluorescent and luminescent data were normalized to those measured during baseline period.

Western-blot and immunocytochemistry

Cells were incubated with different drugs at 37 ºC. Cells were suspended in a hyposmotic solution (1% Triton X-100, 20 mM NaF, 1mM Na-vanadate and 1mM PMSF, phosphatase and protease inhibitors). Samples were run on a 10% SDS-PAGE and transferred onto nitrocellulose

membrane. Detection of proteins was achieved by using the appropriate primary and horse-radish peroxidase-conjugated secondary antibodies. Immunoreactivity was evaluated by densitometry.

In immunocytochemical experiments cells were fixed with 4% paraformaldehide. After permeabilization primary and secondary antibodies were applied for 24 hours and 1 hour, respectively, at 25 °C.

Statistics

Analysis of data was performed using Microsoft Excel, Statistica, Origin or SigmaPlot software. Paired and unpaired Student's t-test, factorial ANOVA, Mann-Whitney U-test and Tukey's post-hoc test were used as appropriate.

Results

Dependence of mitochondrial Ca^{2+} uptake on HCMD

In H295R cells, $[Ca^{2+}]_m$ and the ER-mitochondrion distance were simultaneously monitored with confocal microscopy using green fluorescent protein (GFP) targeted to the outer surface of the endoplasmic reticulum (ER). This approach revealed strong correlation between mitochondrial Ca^{2+} uptake rate and the ER-proximity during angiotensin II (AII)-evoked Ca^{2+} signalling. Mitochondria close to the ER took up Ca^{2+} faster than those far from the Ca^{2+} source. Not unexpectedly, no correlation between ER-mitochondrion distance and Ca^{2+} uptake rate could be observed during K^+-evoked voltage-dependent Ca^{2+} influx. Moreover, Ca^{2+} uptake rate of mitochondria was fully independent from the spatial relationship between mitochondria and plasma membrane in this case. These data suggest that the fast and effective Ca^{2+} uptake into ER-close mitochondria is due to the presence of HCMD between the Ca^{2+} source and the opposing mitochondrion whereas Ca^{2+} uptake during Ca^{2+} influx occurs without the formation of such microdomains.

In accordance with H295R cells we were also able to detect HCMD-independent mitochondrial Ca^{2+} uptake in COS-7 cells. Using total internal reflection (TIRF) microscopy we monitored $[Ca^{2+}]_m$ in the $\leq$ 100 nm subplasmalemmal space during capacitative Ca^{2+} entry. To label store-operated Ca^{2+} influx channels STIM-1, a protein of the influx complex, was tagged with red fluorescent protein. Kinetics of mitochondrial Ca^{2+} uptake was not dependent on the distance between mitochondria and the store-operated channels indicating that ion uptake occurred in a HCMD-independent manner.

Inhibitory effect of angiotensin II on mitochondrial Ca^{2+} uptake in H295R cells

Ca^{2+} uptake rate into mitochondria far from ER vesicles was slower during AII stimulation than during K^+-induced signalling. Knowing that the two agonists evoke comparable "global" $[Ca^{2+}]_c$-elevation, this suggests that AII not only generates cytosolic and thus mitochondrial Ca^{2+} response but also attenuates Ca^{2+} uptake into the organelle. We used the broad spectrum phosphatase inhibitor okadaic acid to test the possible involvement of phosphorylation in this inhibition. Okadaic acid significantly reduced the efficiency of mitochondrial Ca^{2+} uptake in AII-stimulated cells suggesting that AII attenuates Ca^{2+} uptake in a phosphorylation-dependent manner. (Ratio of mitochondrial to cytosolic Ca^{2+} peak was used to characterize Ca^{2+} uptake efficiency.)

Okadaic acid reduced the first peak of AII-evoked mitochondrial Ca^{2+} response implying that AII-mediated inhibition was already activated *before* the Ca^{2+} release. This suggests that the inhibitory mechanism can be activated independently from the AII-induced Ca^{2+}signal. Next, we tested this assumption. After the depletion of intracellular Ca^{2+} stores (and still in the absence of extracellular Ca^{2+}) AII was added. Under such conditions AII failed to bring about any $[Ca^{2+}]_c$ change. However, AII was able to attenuate mitochondrial Ca^{2+} uptake during subsequent store-operated Ca^{2+} entry clearly showing that activation of the inhibitory mechanism does not require Ca^{2+} response.

p38 mitogen activated kinase (p38 MAPK) is a protein that can be activated by AII in a Ca^{2+} independent manner in H295R cells. Our Western-blot experiments also revealed phosphorylation and thus activation of p38 MAPK within one minute upon AII-addition. Moreover, pharmacological inhibition (SB202190 or PD169316) of p38 MAPK as well

as knock-down with siRNA both increased the efficiency of mitochondrial Ca^{2+} uptake in AII-stimulated cells. (The knock-down effect of siRNA on p38 MAPK expression was confirmed with immunocytochemistry.) These data show that p38 MAPK is an absolute prerequisite for AII-mediated inhibition of Ca^{2+} uptake into mitochondria of H295R cells. Kinetic analysis of AII-evoked Ca^{2+} signal together with that of p38 MAPK phosphorylation strongly suggest that activation of the kinase precedes Ca^{2+} release.

Pharmacological inhibition or knock-down of p38 MAPK abolished the strong correlation between Ca^{2+} uptake rate and ER-mitochondrion distance suggesting that AII inhibits Ca^{2+} uptake predominantly into ER-far mitochondria.

Effect of *novel-type* PKC isoforms on mitochondrial Ca^{2+} uptake

Although inhibition of p38 MAPK increases Ca^{2+} uptake into mitochondria activation of the kinase with TNFα or with *S. aureus* α-toxin alone has no effect on Ca^{2+} uptake efficiency during K^+-evoked voltage-dependent Ca^{2+} entry.

AII is capable of activating not only p38 MAPK but also a *novel-type* PKC isoform (PKCε) in a Ca^{2+}-independent way in H295R cells. We used a pharmacological approach in order to test the possible involvement of *novel-type* PKC isoforms (nPKC) in the inhibition of mitochondrial Ca^{2+} uptake. Conventional and *novel-type* PKC isoforms were both activated with bisindolyl-maleimide (BIS) and the conventional isoforms were inhibited with Gö6976 at the same time. (With these means nPKC can be *separately* activated.) As in the case of p38 MAPK, activation of nPKC alone did not affect the efficiency of mitochondrial Ca^{2+} uptake. On the other hand, simultaneous activation of p38 MAPK and nPKC with BIS + Gö6976 + TNFα attenuated Ca^{2+} uptake into mitochondria during K^+-

evoked Ca^{2+} signal. Thus, *simultaneous* activation of the kinases can mimic the inhibitory effect of AII.

The role of mitochondrial outer membrane in the control of Ca^{2+} uptake

The attenuation of mitochondrial Ca^{2+} uptake was independent form the source of Ca^{2+}. Thus it was reasonable to presume that the kinases affect Ca^{2+} uptake via a mitochondrial site of action rather than via the modulation of Ca^{2+} access to the organelle. Decreased $\Delta\Psi_m$ (i.e. reduced driving force through the inner mitochondrial membrane) or reduced Ca^{2+} permeability of the outer membrane (i.e. limited Ca^{2+} availability of the MCU in the inner membrane) could both account for attenuated Ca^{2+} uptake into mitochondria.

To assess the role of $\Delta\Psi_m$ and outer mitochondrial membrane (OMM) in the action of the kinases H295R cells were investigated in two groups. In the kinase activated group cells were pre-incubated with TNFα + BIS + Gö6976 in order to simultaneously activate p38 MAPK and nPKC. Kinase inhibited cells were transfected with siRNA against p38 MAPK and exposed to inactive analogues. Using this approach we intended to enhance the contrast between the groups in terms of kinase activity.

In permeabilized H295R cells (after substrate withdrawal-induced depolarization) reduction of extramitochondrial $[K^+]$ in the presence of the K^+ ionophore valinomycin induced an outward K^+ current that (re)polarized mitochondria. This newly formed membrane potential was indistinguishable in the two groups; however Ca^{2+} (5 μM)-evoked Ca^{2+} uptake rate was significantly slower in the kinase activated group. This finding was further confirmed by the next experiment in which mitochondria were fully depolarized by substrate withdrawal plus the addition of protonophore, ATP-synthase inhibitor and respiratory chain inhibitor. Again, the rate of

Ca^{2+}-evoked Ca^{2+} uptake was attenuated by kinase activation. Since concentration gradient is the only driving force for Ca^{2+} uptake under such conditions, these data unambiguously show that the kinase-mediated inhibition of mitochondrial Ca^{2+} uptake is independent from $\Delta\Psi_m$.

In order to study the role of OMM in mediating the inhibition of Ca^{2+} uptake, the outer membrane was permeabilized. Permeabilization was achieved with the cleaved form of the proapoptotic protein Bid (t-Bid). Exposure of permeabilized H295R cells to t-Bid resulted in mitochondrial depolarization due to loss of cytochrome c. However, the fluorescence of matrix-entrapped calcein was unaffected implying that t-Bid selectively permeabilized the OMM. t-Bid had no affect on Ca^{2+} uptake rate in kinase inhibited cells. On the other hand, disruption of the OMM completely abolished the inhibition of Ca^{2+} uptake in the kinase activated group. (Application of hyposmotic shock for the permeabilization of the OMM gave results identical with those obtained with t-Bid.) These findings strongly suggest that OMM in fact can limit mitochondrial Ca^{2+} uptake in a kinase-dependent manner.

Effect of cytosolic Mg^{2+} on mitochondrial Ca^{2+} uptake

Mg^{2+} is regarded as a general inhibitor of Ca^{2+} transport processes but its role as a physiological controlling factor is ambiguous. Therefore we assessed the effect of Mg^{2+} on mitochondrial Ca^{2+} uptake in the $[Mg^{2+}]$-range typical for the cytoplasm of intact cells. $[Ca^{2+}]_m$ was measured with mitochondria-targeted aequorin in permeabilized HEK-293T cells. Ca^{2+} uptake and subsequent efflux were evoked by changing the $[Ca^{2+}]$ of the cytosol-like medium at different concentrations of Mg^{2+}. (These experiments were conducted at 25 °C.)

Mg^{2+} attenuated Ca^{2+} uptake rate in the 0.25-2 mM range dose-dependently. The rate of Ca^{2+} efflux was unaffected. The effect of Mg^{2+} was most pronounced in the 0.25-0.5 mM [Mg^{2+}]-range and in the 500-1000 nM [Ca^{2+}]-range. The inhibition was reversible, repeatable and had no measurable lag time. Thus, physiological concentrations of Mg^{2+} are capable of reducing mitochondrial Ca^{2+} uptake.

Cytosolic Mg^{2+} signal and the identification of its sources

In light of previous findings the most important question was whether or not there are significant changes in cytosolic [Mg^{2+}] ([Mg^{2+}]$_c$) that could influence mitochondrial Ca^{2+} uptake in the intact cell. Stimulating HEK-293T cells with the P_{2y} agonist ATP brought about a cytosolic Ca^{2+} signal with an amplitude of 0.8-1 μM amplitude. This Ca^{2+} signal was accompanied by a simultaneous Mg^{2+} response during which [Mg^{2+}]$_c$ rose form the resting 0.3 mM up to 0.7-0.8 mM. Thus, in intact HEK-293T cells IP_3-mediated Ca^{2+} and Mg^{2+} signals are both in the concentration-range in which mitochondrial Ca^{2+} uptake displays the highest Mg^{2+} sensitivity. (Store-operated Ca^{2+} signal, although its amplitude was similar to that evoked by ATP, failed to induce notable increase in Mag-Fluo-4 fluorescence. Thus, in the Ca^{2+} and Mg^{2+} concentration-range typical for intact HEK-293T cells, Mag-Fluo-4 predominantly reflects [Mg^{2+}].)

Hydrolysis of intracellular ATP automatically results in elevated [Mg^{2+}]$_c$. However, IP_3-mediated Ca^{2+} signal and ATP hydrolysis displayed entirely different kinetics inasmuch as the former was 50 times faster. ATP hydrolysis therefore cannot account for the IP_3-mediated fast Mg^{2+} signal.

UV-induced photolysis of "caged-Ca^{2+}" also brought about an elevation in [Mg^{2+}]$_c$ indicating that Ca^{2+} itself may displace Mg^{2+} from its cytosolic binding sites. However, even if the photolysis-evoked and the IP_3-

mediated cytosolic Ca^{2+} signals were of similar amplitude the latter was accompanied by a higher Mg^{2+} response. This suggests that IP_3 *releases* also Mg^{2+} from an internal store. We tested this assumption in permeabilized HEK-293T cells by simultaneously measuring $[Ca^{2+}]_c$ and $[Mg^{2+}]_c$ during stimulation with IP_3. Under such conditions IP_3 released not only Ca^{2+} but also Mg^{2+} from an internal store.

Conclusions

We have shown that AII has a dual effect on mitochondrial Ca^{2+} homeostasis in H295R cells. By releasing Ca^{2+} it primarily induces mitochondrial Ca^{2+} response. AII-evoked *simultaneous* activation of p38 MAPK and PKC isoforms attenuates Ca^{2+} uptake into the organelle. The inhibition is apparent even in the low submicromolar $[Ca^{2+}]$-range and is most probably mediated by the reduction of Ca^{2+} permeability of the outer mitochondrial membrane. Thus, the outer mitochondrial membrane is a regulating (and regulated) factor of mitochondrial Ca^{2+} uptake.

Simultaneous activation of p38 MAPK and nPKC isoforms inhibits Ca^{2+} uptake of the whole mitochondrial population in the cell. However, in the case of Ca^{2+} release to ER-near mitochondria this inhibition can be effectively antagonized by the high $[Ca^{2+}]$ in the HCMD. Mitochondrial Ca^{2+} uptake during AII-stimulus is therefore shaped by both the ER-mitochondrion distance and p38 MAPK/nPKC activity. The effect of the kinases on Ca^{2+} uptake can be regarded as a feed-forward inhibition. On the other hand, during voltage-dependent Ca^{2+} influx and (in COS-7 cells) during store-operated Ca^{2+} entry lack of kinase activation allows mitochondrial Ca^{2+} uptake to occur without the formation of HCMDs.

We have demonstrated in HEK-293T cells that cytosolic Ca^{2+} signal is accompanied by a simultaneous Mg^{2+} response. Both signals are in the concentration-range in which mitochondrial Ca^{2+} uptake displays the highest Mg^{2+} sensitivity. Hydrolysis of cytosolic ATP does not notably contribute to the initial fast phase of the Mg^{2+} signal. In fact, displacement of Mg^{2+} by Ca^{2+} and IP_3-mediated Mg^{2+} release are the major sources of the Mg^{2+} response. Thus, cytosolic Mg^{2+} may be a regulating factor of mitochondrial Ca^{2+} homeostasis also in the intact cell.

List of publications

PhD thesis is based on the following publications:

***Szanda G**, Koncz P, Várnai P, Spät A.*
Mitochondrial Ca^{2+} uptake with and without the formation of high-Ca^{2+} microdomains
Cell Calcium,40:527-37,2006
(I.f.: 4,118)

***Szanda G**, Koncz P, Rajki A, Spät A.*

Participation of p38 MAPK and a novel-type protein kinase C in the control of mitochondrial Ca^{2+} uptake

Cell Calcium, 43:250-9, 2008

(I.f.: 4,481)

***Szanda G**, Rajki A, Gallego-Sandín S, Garcia-Sancho J, Spät A.*
Effect of cytosolic Mg^{2+} on mitochondrial Ca^{2+} signaling
Pflugers Arch. (Eur.J.Physiol.) 457: 941-54, 2009
(I.f.:3,526)

*Spät A, Fülöp L, Koncz P, **Szanda G**.*
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Acta Physiol (Oxf)195:139-47,2009
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Cell Calcium, 48:168-75, 2010
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*Korzeniowski MK, **Szanda G**, Balla T, Spät A.*

Store-operated Ca^{2+} influx and subplasmalemmal mitochondria

Cell Calcium 46:49-55, 2009

(I.f.: 4,288)

Publications related to the thesis:

*Pitter JG, **Szanda G**, Duchen MR, Spät A.*
Prostaglandin F2alpha potentiates the calcium dependent activation of mitochondrial metabolism in luteal cells
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High- and low-calcium-dependent mechanisms of mitochondrial calcium signaling

Cell Calcium, 44:51-63, 2008 (I.f.: 4,481)

*Koncz P, **Szanda G**, Fülöp L, Rajki A, Spät A.*

Mitochondrial Ca^{2+} uptake is inhibited by a concerted action of p38 MAPK and PKD

Cell Calcium 46:122-129, 2009 (I.f.: 4,288)