Ca$^{2+}$ and cAMP cross-talk in mitochondria

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Abstract

While mitochondrial Ca\(^{2+}\) homeostasis has been studied for several decades and many of the functional roles of Ca\(^{2+}\) accumulation within the matrix, at least in part, clarified, the possibility of modulation of the organelle functions by cAMP remains largely unknown.

In this contribution we briefly summarize the key aspects of Ca\(^{2+}\) and cAMP signalling pathways in mitochondria. In particular, we focus on recent findings concerning the discovery of an autonomous cAMP toolkit within the mitochondrial matrix and its role in controlling mitochondrial ATP synthesis. A description of the main methods presently available to measure Ca\(^{2+}\) and cAMP in mitochondria of living cells with genetically encoded probes is also presented.
Introduction

During the second half of the last century, mitochondria have been extensively studied and their major biochemical functions have been firmly established: the chemiosmotic theory and their prominent role as the “powerhouse of the cell” are now universally accepted (Drago et al., 2011). More recent data have conclusively demonstrated that mitochondria form in the cytoplasm an elaborate network in a constant dynamic state, due to continuous processes of fission and fusion (Scorrano, 2007) as well as that individual organelles move along the cytoskeleton towards specific cellular locations (MacAskill & Kittler, 2010). The two membranes surrounding mitochondria have different protein and lipid composition and functional roles: the outer membrane (OMM) is highly permeable to solutes up to 5 kDa while the inner membrane (IMM) is impermeable to ions and to most hydrophilic molecules (but for the presence of specific transporters) and hosts the proteins of the respiratory chain (RC) and the ATP synthase. The IMM is composed by cristae, generated by in-folding processes, which expand its surface and enhance its capacity of hosting, in a narrow space, a large number of RC complexes and ATP synthetizing enzymes. In terms of second messenger signalling molecules, the OMM, the inter-membrane space and the outer surface of the IMM are believed to experience the same concentrations existing in the cytoplasm, while the inner surface of the IMM and the matrix are believed to be totally insulated from cytoplasmic second messengers (with the notable exception of Ca$^{2+}$).

While Ca$^{2+}$ signalling in mitochondrial patho-physiology has been thoroughly studied and its prominent role is now largely accepted, the existence of a cAMP signalling around and inside mitochondria has just started to be unravelled. In the cytosol, the signalling systems of these two major second messengers can cross-talk, interacting at different levels (Bruce et al., 2003). For example, activation of G-protein coupled receptors (GPCRs) linked to either G$_q$ and G$_i$/G$_s$ proteins impact on both Ca$^{2+}$ and cAMP cytosolic concentration. Moreover, Ca$^{2+}$ can modulate components of the cAMP signalling toolkit, such as adenylate cyclases (ACs) and phosphodiesterases (PDEs);
in turn, PKA, the main cAMP target, can modulate the release of Ca\(^{2+}\) from intracellular stores, through regulation of IP\(_3\)-receptors (IP\(_3\)Rs) and ryanodine-receptors (RyRs), as well as Ca\(^{2+}\) clearance, through modulation of the Ca\(^{2+}\) pumps, SERCA and PMCA. New findings on the role of cAMP in mitochondria physiology discloses new contact points in the cross-talk between these two master cell regulators.

**Ca\(^{2+}\) signalling**

The capacity of mitochondria to accumulate Ca\(^{2+}\) in their matrix in an energy dependent process was first unravelled in the late 60’s and the regulation by Ca\(^{2+}\) of several key aspects of mitochondria functionality, from enzyme activity to gene expression, has been the topic of intense investigation ever since (McCormack *et al.*, 1990). Mitochondrial Ca\(^{2+}\) influx is driven by the electrical gradient, about -180mV, generated by the electron transport chain (or by the reversal of the ATP synthase) and is mediated by a gated channel named mitochondrial Ca\(^{2+}\) uniporter, MCU; the efflux of Ca\(^{2+}\) from the matrix into the surrounding cytoplasm, is mediated by the xNa\(^+\)/Ca\(^{2+}\) exchanger (mNCX) and the H\(^+\)/Ca\(^{2+}\) exchanger (mHCX) (Figure 1). The molecular nature of MCU and mNCX has been only recently unravelled (Rizzuto *et al.*, 2012).

The positioning of mitochondria in the vicinity of intracellular Ca\(^{2+}\) channels of the ER or of the plasma membrane can be strategic for their activity regulation (Rizzuto *et al.*, 1998). The low Ca\(^{2+}\) affinity of MCU is particularly relevant in this context, inasmuch as the high concentration of Ca\(^{2+}\) in microdomains of close apposition between mitochondria and the Ca\(^{2+}\) channels allows a rapid and efficient Ca\(^{2+}\) uptake by the organelles; on the other hand, as the microdomains rapidly dissipate by diffusion, it prevents dangerous mitochondria Ca\(^{2+}\) overload. Furthermore, this property confers to mitochondria the capacity of decoding different oscillation frequencies and translating them in separated outcomes, rendering these organelles powerful signals integrators (Rizzuto *et al.*, 2012). Indeed mitochondria contribute, together with other organelles, to the generation of the
spatio-temporal complexity of cytosolic Ca\(^{2+}\) signalling. Under basal condition mitochondrial Ca\(^{2+}\) concentration, [Ca\(^{2+}\)]\(_{m}\), is around 50-100 nM, \textit{i.e.}, similar to that in the cytosol, but it can reach values &gt;100 \(\mu\text{M}\) upon strong cell stimulation (Rizzuto & Pozzan, 2006; Rizzuto et al., 2012).

In addition to the role of mitochondrial Ca\(^{2+}\) handling in shaping and modulating the dynamics of [Ca\(^{2+}\)]\(_{c}\), at the surface of the OMM and in the inter-membrane space Ca\(^{2+}\) can modulate the activity of enzymes and transporters, while matrix Ca\(^{2+}\) is essential for the control of specific mitochondrial functions, the best known of which is the activity of three NADH-linked dehydrogenases (for a recent review see (Pizzo et al., 2012).

Mitochondrial Ca\(^{2+}\) overload causes the opening of a high conductance, non-selective channel, named Permeability Transition Pore, PTP (Figure 1), and this event appears to play a key role in both apoptotic and necrotic cell death. The outcome appears correlated with the extent of the Ca\(^{2+}\) overload, which affects the duration of the PTP opening and thus the residual cell ATP availability (Rasola & Bernardi, 2011). As ATP is required for apoptosis to progress, the PTP may ultimately control the switch between the two forms of cell death.

Recent data from different groups have revealed other roles of Ca\(^{2+}\) in mitochondrial functions, such as regulation of organelle shape and movement (MacAskill & Kittler, 2010), fission/fusion (Westermann, 2010) and autophagy (Cardenas & Foskett, 2012).

**cAMP signalling**

In eukaryotes, cAMP is involved in processes as diverse as hormone signal transduction, memory formation and control of heart beating, to cite just a few, whereas in prokaryotes, where its role has been discovered more recently, it mediates the catabolite repression response and represents a master regulator of microbial virulence, modulating different aspects of host-pathogen interaction.
Despite its broad use since early evolution of life, a possible role of this ubiquitous second messenger in mitochondrial patho-physiology has been neglected until recently. Nowadays, the presence of PKA anchoring proteins (AKAPs) on the OMM (Figure 1) is firmly established (Carlucci et al., 2008) and the phosphorylation of OMM proteins by cytosolic PKA is known to affect important functions of the organelles. For example, PKA anchored to the OMM through the AKAP WAVE-1, and possibly also through D-AKAP1 and Rab32 (Bui et al., 2010), is thought to be an important checkpoint for apoptosis regulation, mediating the phosphorylation and inactivation of the pro-apoptotic protein BAD (Danial et al., 2003). In addition, cAMP anti-apoptotic effects are also mediated by the cAMP effector Epac (exchange protein directly activated by cAMP) (Insel et al., 2012a). On the other hand, cAMP has been also proposed as an apoptosis inducer, see for example (Insel et al., 2012b). In particular, it has been shown that cAMP generated by the soluble form of adenylate cyclase (sAC), through PKA phosphorylation, determines the translocation of the pro-apoptotic Bax to mitochondria (Appukuttan et al., 2012).

Cytosolic PKA also modulates mitochondrial shape and autophagic degradation by phosphorylating the pro-fission protein Drp1; indeed, phosphorylated cytosolic Drp1 does not translocate to the organelles, leaving mitochondrial fusion unopposed (Cribbs & Strack, 2007; Gomes et al., 2011). The balance between Drp1 phosphorylation by PKA (but also by CaMKIα and PKCδ) and dephosphorylation due to either calcineurin, CN, or protein phosphatase 2A, PP2A, impacts, through the regulation of mitochondrial morphology, on other complex events such as autophagic degradation (Gomes et al., 2011) and the regulation of neuronal development (Dickey & Strack, 2011). Finally, it has recently been reported that, through the action of ChChd3, a protein located in the IMM and associated to the AKAP SKIP, PKA regulates mitochondrial functions by coordinating mitochondrial protein import (Darshi et al., 2011).

Given that the cytosolic surface of the OMM is freely accessible not only to cAMP, but also to cytosolic PKA, most of the above described effects are perfectly consistent with the accepted
model of cAMP signalling. On the contrary, though cAMP should be freely permeable through the OMM pores and thus its concentration in the inter-membrane space similar, if not identical, to that in the cytosol, a role for cAMP and PKA-dependent phosphorylation of targets located in the IMM appears more problematic, as: i) the PKA catalytic and regulatory subunits should be impermeable to the OMM and ii) no mitochondrial targeting sequence capable of translocating PKA into the inter-membrane space are known. Even more puzzling is the possibility that cAMP can modulate mitochondrial proteins located within the matrix. Indeed, for many years the general consensus was that cAMP had no role within the organelles. This conclusion was based not only to the absence of targeting sequences in either of the known cAMP effectors, PKA and Epac, but also on the impermeability of cAMP across the IMM and on the plasma membrane exclusive localization of classical G protein-regulated adenylate cyclases (tmACs).

In the last years, however, a number of reports suggested the existence of a cAMP signalling system inside mitochondria. A first study, making use of a FRET-based cAMP sensor in live cells (DiPilato et al., 2004), concluded that cAMP produced in the cytoplasm rapidly diffuses into the organelles; on the contrary, Acin-Perez and colleagues suggested that the IMM is impermeable to cAMP, while the organelles are endowed with a cAMP-PKA-PDEs signaling cascade wholly contained in their matrix (Acin-Perez et al., 2009). In particular, though the intra-mitochondrial level of cAMP was not directly measured, they suggested that cAMP is generated inside the matrix by a mitochondrial form of sAC (Figure 1) in response to bicarbonate, either added exogenously or derived from the carbon dioxide produced by the Krebs cycle, through the action of carbonic anhydrase.

Very recently, making use of a FRET-based cAMP sensor selectively localized to the mitochondrial matrix, we directly demonstrated, in living cells, that cytosolic cAMP does not diffuse from cytosol into the organelles, but instead, consistent with the suggestion of Acin-Perez et al., it is generated in the matrix by a sAC. More importantly, we showed that cAMP increases
within the organelles not only in response to bicarbonate, but also upon increases in matrix Ca\(^{2+}\) levels (Figure 2), in line with the known properties of sAC (Di Benedetto et al., 2013). Indeed sAC is phylogenetically related to prokaryotic cyclases and is synergistically activated \textit{in vitro} by bicarbonate and Ca\(^{2+}\). Thus, sAC resembles tmACs as, similarly to the latter, its activity is modulated by Ca\(^{2+}\), but differs from them as sAC is totally insensitive to heterotrimeric G proteins and forskolin.

Which are the consequences of a matrix cAMP rise and what about its molecular target? As to the first issue, Acin-Perez et al. (Acin-Perez et al., 2009; Acin-Perez et al., 2011) concluded that the main effect of intra-mitochondrial cAMP increase is the activation of the RC and, thus, of ATP synthesis by the organelles. Using a mitochondria-targeted luciferase and a set of genetic and pharmacological tools, we recently directly showed in live cells that cAMP in the matrix indeed contributes positively to the regulation of mitochondrial ATP production (Di Benedetto et al., 2013). Whether or not other mitochondrial functions are regulated by matrix cAMP remains to be investigated.

Rather puzzling, on the contrary, remains the molecular target of matrix cAMP. According to Acin-Perez et al., cAMP generated in the matrix activates a mitochondrial PKA, leading to the phosphorylation and activation of subunit IV-I of cytochrome oxidase (COX), and possibly of other enzymes of the RC, thus enhancing the efficacy of oxidative phosphorylation (OXPHOS). However, other previous data demonstrated that PKA-dependent phosphorylation of COX inhibits OXPHOS (Robin et al., 2003; Helling et al., 2008). PKA has also been reported to increase the activity of complex I (NADH dehydrogenase), phosphorylating several of its subunits (for example see (Papa et al., 2008) and modulating their import and assembly (De Rasmo et al., 2008; Papa et al., 2008). However, a clear demonstration that the PKA responsible for these phosphorylation events resides in the matrix, or in the inter-membrane space, of mitochondria is still lacking. In particular, the key, and still unanswered question, is whether, and through which mechanism, PKA
can enter into the mitochondrial matrix. The presence of PKA associated with isolated mitochondria has been reported several times (see for example (Sardanelli et al., 2006)), but the data concerning its localization in the matrix are still contradictory. The most direct evidence, i.e., the immuno-localization of PKA within mitochondria, depends on the specificity of the antibodies used and the possibility of a cross-reaction with other antigens has not been completely ruled out. Given that the mechanism of mitochondrial protein import excludes the possibility that the mature, folded, PKA subunits can penetrate in the matrix of intact organelles, one has to assume the existence of hidden targeting sequences in PKA. To the best of our knowledge, no evidence for, or against, this possibility has been provided so far. Transient expression of GFP-tagged PKA subunits did not revealed a significant accumulation of PKA into mitochondria (Di Benedetto, unpublished). The possibility therefore cannot be at the moment excluded of the presence in the mitochondrial matrix of a PKA-like enzyme, in turn responsible for the cAMP-dependent modifications of mitochondrial functions reported by us and others. Along these lines, the pharmacological sensitivity of the cAMP-induced increase in mitochondrial ATP only in part overlaps that of cytosolic PKA, further stressing the need for more accurate and unbiased investigation of this problem (Di Benedetto et al., 2013).

It needs also stressing that reversible phosphorylation is emerging as a central mechanism in the control of mitochondrial function, and recently a phospho-proteomic study on human skeletal muscle revealed the presence of tens of mitochondrial phosphoproteins, some of which are potential target of PKA (Zhao et al., 2011).

Conclusions

A unique and critical feature of signal transduction controlled by second messengers is the convergence of a very large variety of extracellular stimuli onto a limited number of intracellular molecules, i.e., Ca^{2+}, cAMP and cGMP and a few others (IP$_3$, NAADP or cADP ribose), the latter linked to Ca^{2+} mobilization. This situation requires an elaborate signalling code, relying not only on
concentration, but also on complex spatio-temporal variations of the second messenger, with
different outcomes depending on its oscillation frequency and sub-cellular localization. cAMP and
Ca\(^{2+}\) reciprocally influence each other, in some case with antagonistic roles and in others
synergistically. Our recent study, together with those of Manfredi’s group, reveal that mitochondria
can start to be regarded as signalling hubs, involving not only Ca\(^{2+}\), as well established since a long
time, but also cAMP. For example, in cardiomyocytes stimulated with agents that augment the
frequency and amplitude of the spontaneous Ca\(^{2+}\) oscillations in the cytosolic and mitochondrial
compartment, matrix cAMP increases (Figure 2). Notably, although the Ca\(^{2+}\) changes in the
mitochondrial matrix are oscillatory and each spike lasts 300-500 ms, the organelle cAMP increases
take a few tens of seconds to reach a new plateau level, suggesting that the intra-mitochondrial
cAMP homeostatic machinery is capable of integrating an oscillatory Ca\(^{2+}\) signal into a prolonged
cAMP increase (Di Benedetto et al., 2013).

The discovery of a cAMP signalling cascade in mitochondria, and the demonstration that
changes in mitochondrial matrix Ca\(^{2+}\) concentration results in modulation of cAMP production,
open the way to a re-evaluation of these organelles not only as key controller of Ca\(^{2+}\) signalling, but
also as integrators of multiple second messengers.
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BOX: Tools for Ca\(^{2+}\) and cAMP dynamic measurements in living cells

Ca\(^{2+}\)

The possibility of measuring the changes in \([\text{Ca}^{2+}]_m\) of living cells started in 1992 with the beginning of the genetically encoded indicators era: the Ca\(^{2+}\)-sensitive photoprotein aequorin was engineered by inserting at its N-terminus a classical mitochondrial targeting sequence that directed it to the mitochondrial matrix (Rizzuto et al., 1992). At the same time, the gene of the green fluorescent protein (GFP) was cloned and a few years later it was demonstrated that GFP can be expressed (and maintained its fluorescence) when transfected in different cells and organelles (Rizzuto et al., 1995). In 1997 Miyawaki et al. (Miyawaki et al., 1997) and Persechini et al. (Persechini et al., 1997) generated the first, GFP based, Ca\(^{2+}\) indicators. These discoveries represent a breakthrough in the cellular imaging field, opening the possibility of studying spatially and temporally regulated dynamics not only of Ca\(^{2+}\), but also of virtually all second messengers. Indeed, in the last 20 years the cellular imaging field has been exponentially growing, producing a variety of genetically encoded indicators of different molecules (Ca\(^{2+}\), cAMP, cGMP, NO, ATP, IP\(_3\), ROS…), with different sensitivity, subcellular localization and different working principles (bioluminescence, changes in intensity or in wavelength of the emitted fluorescence upon binding of the target molecules, FRET, BRET, FLIM, BiFC, CALI).

The first reliable sensor for Ca\(^{2+}\) in the mitochondrial matrix, mt-Aequorin, undergoes, upon Ca\(^{2+}\) binding, a conformational change, causing the oxidation of the cofactor coelenterazine with the emission of a photon. The rate of photon emission is proportional to the \([\text{Ca}^{2+}]\) and, given that photon emission is an irreversible process, aequorin is “consumed” while measuring Ca\(^{2+}\). It is however possible to infer the \([\text{Ca}^{2+}]\) through an appropriate calibration procedure that takes into account the rate of photon emission at any given time as a function of the residual aequorin in the sample. Ca\(^{2+}\) measurements using aequorin do not require external illumination, thus ensuring an excellent signal-to-noise ratio with no photobleaching and no detectable phototoxicity. The main
disadvantage of aequorin-based probes is that the absolute amount of photons emitted by aequorin is low (maximum 1 photon/molecule, in practice much less, i.e., <0.3 photons/molecule); moreover, as mentioned above, each molecule can perform only one emission cycle, so that the amount of responsive probe progressively decreases with time. In addition, given the steep dependence of aequorin emission rate on $[\text{Ca}^{2+}]$ (>100 fold x 10 fold change in $[\text{Ca}^{2+}]$) the average signal of aequorin is dominated by the most responding cells/organelles. Although a number of studies have been published with recombinant aequorin in single cells, the most common application of this probe is in measurement of cell populations. Today, different versions of the aequorin-based mitochondrial sensor exist, with different localization (matrix, OMM, IS) and sensitivity.

Genetically encoded indicators based on GFP (and its mutants) appeared first in 1997, and greatly developed in the following years. They basically consist of a $\text{Ca}^{2+}$-binding domain, from calmodulin or troponin C, fused with one or two GFP variants. When the fluorophore is only one, the binding of $\text{Ca}^{2+}$ alters the chromophore environment, inducing a change in its fluorescence emission intensity (e.g., Pericam, CaMgaroo, GCaMP). On the other hand, two-fluorophores indicators are based on fluorescence resonance energy transfer (FRET): usually FRET is minimum when the $[\text{Ca}^{2+}]$ is low and it increases upon $\text{Ca}^{2+}$ rises. In practical terms, the changes in $[\text{Ca}^{2+}]$ can be dynamically monitored by measuring the ratio between the fluorescence intensity emitted by the acceptor fluorophore and that emitted by the donor fluorophore (upon excitation of the donor). At low $\text{Ca}^{2+}$ the ratio is low but, upon $\text{Ca}^{2+}$ binding to the sensor, a conformational change occurs that brings the two fluorophores in closer proximity, FRET increases and the ratio increase (e.g., Cameleons; (Rudolf et al., 2003)). In the last years many improvements have been made to the original probes: fluorophores have been replaced or modified to decrease the sensors sensitivity to pH, to $[\text{Cl}^{-}]$, and to photobleaching, and to improve the dynamic range of FRET changes upon $\text{Ca}^{2+}$ binding. In addition, cameleons have been modified in order to minimize the interference with endogenous calmodulin targets (Palmer et al., 2006), and to improve the specificity of their localization. As for aequorin, different cameleons have been generated with variable $\text{Ca}^{2+}$ affinities.
(Palmer et al., 2006). As far as mitochondria are concerned, cameleons targeted to the matrix or to the cytoplasmic surface of the OMM are available (Giacomello et al., 2010)).

**cAMP**

The study of intracellular cAMP dynamics suffered from the total lack of dedicated sensors until the early 1990s, so that the only possible cAMP measurements until then were in cell populations with biochemical methods. For this reason the field of cAMP dynamics in living cells developed much later in comparison with the study of Ca$^{2+}$ dynamics. cAMP sensors were generated taking advantage of the cAMP dependent conformational changes in its natural targets. In particular, cAMP is known to activate three different types of downstream effectors: cyclic nucleotide gated channels (CNGCs), Epac, and PKA; all these effectors have been exploited in developing cAMP sensors (for review see (Willoughby & Cooper, 2006))

CNGCs are plasma membrane cations channels activated upon binding of cyclic nucleotides. They have been used as indirect means to measure cAMP concentration in the proximity of the plasma membrane; cyclic nucleotides binding to channels activate an inward cation current ($I_{CNG}$), that can be measured directly by electrophysiological methods and/or indirectly by monitoring the associated increases in intracellular [Ca$^{2+}$]. The main limitation (but also advantage) of this approach is that, apart from the low selectivity for cAMP versus cGMP, the measure is confined to the vicinity of the plasma membrane.

The first intracellular probe allowing a direct measure of cAMP (FiCRhR) in the cytosol of single live cells was a FRET-exploiting sensor based on PKA (Adams et al., 1991). This probe, however, was not genetically encoded, but the regulatory (R) and catalytic (C) PKA subunits were chemically labelled with fluorescein and rhodamine, respectively, and then microinjected in living cells. When a genetically encoded version of such a sensor appeared (Zaccolo et al., 2000), with chimeric R and C subunits fused to GFP variants, it opened the way to the study of intracellular cAMP dynamics in living cells with a much simpler technique, pioneering the understanding of
cAMP signalling in live heart cells (Zaccolo & Pozzan, 2002). This PKA-based sensor uses the same principle developed for Cameleons, *i.e.*, FRET, but in this case the fluorescence changes work in the opposite way: when cAMP is low, the C and R PKA subunits are in close proximity, and FRET is maximum; when cAMP increases, the two subunits dissociate and FRET decreases. The main disadvantages of the PKA-based probe are its possible interference with endogenous PKA signalling and its relatively slow kinetics. To overcome these drawbacks, a few years later a simpler single-chain PKA-based FRET sensor, without catalytic properties and with faster kinetics, was developed (Willoughby & Cooper, 2006).

In 2004 the first Epac-based FRET sensors were generated, which appeared in different “flavours” from distinct laboratories (Willoughby & Cooper, 2006). All of them contain either the full length or only the cAMP binding domains of Epac1 or Epac2 and, together with the subsequently developed variants, they differ in terms of cAMP affinity, catalytic activity, subcellular localization and GFP variants pairs, in turn implying different sensitivity to pH, to [Cl\(^{-}\)], and to photobleaching, and different dynamic range. Generally, they display faster kinetics and higher dynamic range in comparison with the PKA-based sensor, and have became the most popular indicators for imaging cAMP in living cells.

As to cAMP probes targeted to the mitochondria, three variants, all based on Epac, are presently available: i) a sensor, named MitoCOX-ICUE1 (DiPilato *et al.*, 2004), that however is largely mis-targeted to the cytoplasm (Di Benedetto *et al.*, 2013); ii) a sensor, named MitoDAKAP1-ICUE1, targeted to the OMM (DiPilato *et al.*, 2004), and iii) a sensor, named 4mtH30, with multiple mitochondrial targeted sequences, that is completely trapped within the matrix (Di Benedetto *et al.*, 2013).
Figure Legends

Figure 1. Mitochondrial Ca\(^{2+}\) and cAMP toolkit.

The cartoon shows the main molecules involved in mitochondrial Ca\(^{2+}\) and cAMP handling. In particular for Ca\(^{2+}\) influx: i) the Voltage-Dependent Anion Channel, VDAC. ii) the Mitochondrial Calcium Uniporter, MCU; iia) MICU1 (mitochondrial Ca\(^{2+}\) uptake protein 1); iib) MICU2 (mitochondrial Ca\(^{2+}\) uptake protein 2); iic) MCUR1 (mitochondrial calcium uniporter regulator 1). For Ca\(^{2+}\) efflux: i) mNCX (xNa\(^{+}\)/Ca\(^{2+}\) exchanger); ii) mHCX (Ca\(^{2+}\)/H\(^{+}\) exchanger).

As far as cAMP is concerned, within the organelle the following proteins are present: i) AKAPs (A Kinase Anchoring Proteins): ii) soluble adenylate cyclase (sAC); ii) phosphodiesterase (PDE); iii) PKA, or a mitochondrial PKA-like enzyme.

Figure 2. cAMP is elicited by Ca\(^{2+}\) increases in the mitochondrial matrix of neonatal rat cardiomyocytes

The representative kinetics of \(\Delta R/R_0\) changes recorded in a rat neonatal cardiomyocyte co-expressing a mitochondrial (4mtH30) and a nuclear (H30_NLS) cAMP sensor are presented. The cell was stimulated, in sequence, with norepinephrine (NE; 10 \(\mu\)M) and forskolin (25 \(\mu\)M). This double stimulation has been performed twice: initially in the absence of Ca\(^{2+}\) (Ca\(^{2+}\)-free medium containing 100 \(\mu\)M EGTA and 30 \(\mu\)M TBQ, the SERCA inhibitor tert-butylhydroquinone, to keep the ER Ca\(^{2+}\) store completely empty), and then after washing the drugs and in the presence of 1 mM CaCl\(_2\) in the medium. For details see Di Benedetto et al., 2013.
References


