FUNCTIONAL ROLE OF MITOCHONDRIAL REACTIVE OXYGEN SPECIES IN PHYSIOLOGY

Plamena R. Angelova and Andrey Y. Abramov

Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London

Corresponding authors:

Dr Plamena R. Angelova and Prof Andrey Y. Abramov, Department of Molecular Neuroscience, Institute of Neurology, University College London, Queen Square House, Queen Square, London, WC1N 3BG, United Kingdom.

Emails: p. stroh@ucl.ac.uk or a.abramov@ucl.ac.uk

ABSTRACT

The major energy generator in the cell – mitochondria produce reactive oxygen species as a by-product of a number of enzymatic reactions and ATP production. Emerging evidence suggests that mitochondrial ROS regulate diverse physiological parameters and that dysregulated ROS signalling may contribute to a development of pathologies which leads to human diseases. ROS produced in mitochondrial enzymes are trigger for monoamine-induced calcium signal in astrocytes, playing important role in physiological and pathophysiological response to dopamine. Generation of ROS in mitochondria leads to peroxidation of lipids, which is considered to be one of the most important mechanisms of cell injury under condition of oxidative stress. However, it also can induce activation of mitochondrial and cellular phospholipases that can trigger variety of the signals – from activation of ion channels to stimulation of calcium signal. Mitochondria are shown to be the oxygen sensor in astrocytes, therefore inhibition of respiration by hypoxia induces ROS production which leads to lipid peroxidation, activation of phospholipase C and induction of IP3-mediated calcium signal. Propagation of astrocytic calcium signal stimulates breathing activity in response to hypoxia. Thus, ROS produced in mitochondrial enzymes or electron transport chain can be used as a trigger for signalling cascades in central nervous system and deregulation of this process leads to pathology.

Introduction

Although mitochondria have a multiple important functions, this organelle is mainly high efficient engine for conversion of the products of glucose or fatty acid metabolism into universal form of energy which is accepted by all types of cells – ATP. As in every highly efficient engine, the process of ATP production in the oxidative phosphorylation coupled with the mitochondrial respiration in the electron transport chain is having a leakage of energy in the form of electrons. These outgoing electrons can hit the nearby situated molecules and ultimately produce free radicals. Mitochondria are using most of the oxygen taken up by the cell and considering this oxygen-rich environment, the production of Reactive Oxygen Species (ROS) is inevitable. Electrons escaping the electron transport chain in the first instance generate ROS mainly in the form of super oxide radical $O_2^{\cdot -}$. This superoxide converts to hydrogen peroxide (Boveris et al., 1972; Loschen et al., 1971; Loschen et al., 1974).

Compared to the activated enzymes such as NADPH or xanthine oxidases mitochondria produce less ROS, but in contrast to any other enzymatic and non-enzymatic ROS producer, this organelle is able to generate free radicals continually (Gandhi and Abramov, 2012). Considering the high reactivity and toxicity of ROS, our cells (and particularly the mitochondria) in the time of evolution have not only developed effective defence systems against oxidative damage but also adopted ROS to play signalling and regulatory role in physiological processes.

The rate of production and the level of ROS produced by mitochondria can be modulated by a number of factors that render this system to be an ideal regulatory instrument in the cellular homeostasis and to be a messenger in the signal transduction cascades.

In the following review we discuss the physiological role of ROS produced in the mitochondria and how deregulation of this process can lead to development of pathology and become trigger of cell death (which is as well one of the multiple mitochondrial functions).

Mitochondrial ROS producers

In the majority of publications under the term "mitochondrial ROS" the authors mean the ROS produced in the electron transport chain (ETC) of mitochondria. ROS in mitochondria can be formed by enzymatic action of numerous enzymes including monoamine oxidase (MAO) and cytochrome b5 reductase (Cb5R) located on the outer mitochondrial membrane, as well as glycerol-3-phosphate dehydrogenase and in some cell types, various cytochrome P450 enzymes located in the inner mitochondrial membrane. There are also several matrix enzymes and complexes including aconitase, pyruvate dehydrogenase (PDH), and α-ketoglutarate dehydrogenase (αKGDH) that can generate superoxide (Finkel, 2011). Superoxide can rapidly dismutate spontaneously or enzymatically with the help of manganese superoxide dismutase (MnSOD). The formed freely permeable H_2O_2 can be further degraded by enzymes such as catalase (CAT), glutathione peroxidase (GPx), and peroxiredoxin 3 (Prx3) (Brand, 2010).

Production of ROS in the ETC

The main source of reactive oxygen species in the mitochondria is the electron leak from the electron transport chain (ETC). Depending on the direction of the electron "escape" from ETC, ROS (mainly superoxide anion; $O_2^{\cdot-}$) is produced in the matrix or between inner and outer membrane of mitochondria (Figure 1). Electron transport between the complexes of the electron transport chain is limited by the availability of substrates for this complexes (NADH and succinate), but also on the rate of production of substrates in the TCA cycle, the rate of consumption of ATP and/or on availability of the final electron acceptor (oxygen) of the electron transport. However, ROS production that is linked to the topology of the electron carriers and their functional integration is determined by the rate constants of the enzymatic reactions of the complexes of the electron transport chain. Moreover, ROS production is dependent on the values of the mitochondrial membrane potential (Starkov and Fiskum, 2003; Votyakova and Reynolds, 2001). The leak of electrons can be induced by inhibition of complex I and III with specific inhibitors (e.g. rotenone and antimycin A), that lead to a higher rate of reactive oxygen production in the mitochondria (Lenaz, 2001; Murphy, 2009). Inhibition of respiration and decrease of mitochondrial membrane potential by anoxia or hypoxia can stimulate ROS production in the same way but only for short time (Abramov et al., 2007; Nohl et al., 1993). High mitochondrial membrane potential also increases a rate of ROS production in ETC and it can be elevated by application of the mitochondrial substrates (Abramov et al., 2010; Kovac et al., 2015). Mild uncoupling with low doses of FCCP or physiological uncouplers significantly reduces production of superoxide and for long time is used as a basis for cell protective strategy (Brennan et al., 2006; Skulachev, 1996). Although the use of chemical uncouplers reduced mitochondrial ROS production, the decrease of oxidative stress in general may be limited because FCCP on the other hand can maximise superoxide production in activated NADPH oxidase (Abramov et al., 2005). Thus, the rate of ROS production in ETC

can be manipulated by changing the mitochondrial membrane potential, using inhibitors and substrates of the mitochondrial enzymatic complexes.

ROS production in the matrix of mitochondria – TCA

It has been confirmed that ROS production in mitochondria takes place as well outside the electron transport chain: isolated α-ketoglutarate dehydrogenase (KGDHC; the rate-limiting enzyme of the TCA cycle) or pyruvate dehydrogenase complexes (PDHC) are producing superoxide or hydrogen peroxide in the absence of ETC complex inhibitors (Starkov et al., 2004). Interestingly, despite generating ROS, α-ketoglutarate dehydrogenase complex is on the same time very sensitive to oxidative stress, e.g. peroxynitrite inhibited purified KGDHC activity in a dose-dependent manner and its activity could be restored by GSH (Shi et al., 2011). KGDHC can be also inactivated by hydrogen peroxide in bovine cardiac mitochondria, fibroblasts or N2 cells (Figure 1). Moreover, inhibition of KGDHC by hydrogen peroxide limits the amount of NADH available to the complex I from the respiratory chain (Tretter and Adam-Vizi, 2000; Tretter and Adam-Vizi, 2004). Reduced activity of brain α-ketoglutarate dehydrogenase complex occurs in a number of neurodegenerative diseases like Parkinson's disease and Alzheimer's disease, where oxidative stress is thought to be central to the pathophysiology of these diseases (Gibson et al., 2003; Gibson et al., 2005; Gibson et al., 2012). Further, in mitochondria the succinate dehydrogenase (SDH) complex as well is capable of superoxide production in the absence of an electron acceptor (Zhang et al., 1998).

ROS produced at the outer membrane of mitochondria

One of the most effective pharmacological targets in neurology is the mitochondrially located (outer membrane) family of enzymes monoamine oxidase (MAO; Figure 1). MAO catabolises dietary monoamines through oxidative deamination, producing aldehydes and H_2O_2 (Hare, 1928). ROS production in the form of hydrogen peroxide can be stimulated by catabolism of monoamines from food or generally in the homeostasis of neurotransmitters and neuromodulators – dopamine, serotonin and adrenaline (Barchas and Freedman, 1963). There are possible intercommunications between ROS from MAO and ETC – deficiency in MAO-A reveals protection against inhibitors of mitochondrial complexes I, III and IV (Fitzgerald et al., 2014).

Regulatory role of mitochondrial ROS

Mitochondrial ROS as activators of phospholipases

Phospholipases play housekeeping and signalling role in the cells. It has been demonstrated that ROS production could activate phospholipases (van Rossum et al., 2004). This activating effect has been shown for different form of ROS including the product of oxidative stress – lipid peroxidation (Hermann et al., 2014; Marzoev et al., 1987; Timusheva et al., 1998). Lipid peroxidation is affecting different isoforms of phospholipase A in mitochondria, cytosol and lysosomes. Stimulating effect of lipid peroxidation has been also shown for phospholipase D (Natarajan and Garcia, 1993) and, importantly for phospholipase C (Domijan et al., 2014; Rossi et al., 1994). We have recently shown that only lipids that are oxidized can be used as a

substrate for the phospholipase C (Domijan et al., 2014). This is a natural process by which damages in the membranes are repaired and further imply a possibly important role of PLC as a part of the cellular antioxidant defence system and the role of ROS and lipid peroxidation in calcium signalling by producing IP3. Confirming this, mutation and loss of phospholipase activity lead to increased lipid peroxidation in mitochondria and cytosol and can be a trigger of neurodegeneration (Kinghorn et al., 2015). Overproduction of lipid peroxidation is described for various types of pathology (Angelova et al., 2015a) playing an important role in cytochrome c release from mitochondria in initiation of cell death (Kagan et al., 2005).

Role of ROS in signal transduction

It has been observed that application of low doses of hydrogen peroxide to the cells induces calcium signal (Domijan et al., 2014; Gonzalez-Pacheco et al., 2002; Granados et al., 2007). Considering this, activation of endogenous ROS formation in mitochondria should play an important role in the initiation of signal transduction. Thus, dopamine-induced calcium signal in astrocytes is due to production of ROS in MAO and it can be blocked by molecular or pharmacological inhibition of this enzyme or by application of antioxidants (Vaarmann et al., 2010). Generation of hydrogen peroxide in MAO stimulates lipid peroxidation, activation of phospholipase C and generation IP_3-dependent calcium signal (Vaarmann et al., 2010) confirming the important role of mitochondrial ROS in signal transduction in CNS.

An important role for ROS has been shown for modulation of various ion channels (BK, KCN, ERG, TRP, etc.) and consequently for the regulation of the neuronal and cardiomyocyte excitability (Angelova and Muller, 2006; Angelova and Muller, 2009; Hool and Corry, 2007; Liu and Gutterman, 2002; Tang et al., 2001). Changes in the intracellular redox milieu can affect many physiological processes, e.g. the gating properties of ion channels and the activity of certain transport mechanism and therefore it could play an essential role in the cellular signal transduction process in a variety of systems. Cysteine or methionine residues on diverse (principal or auxiliary) channel and receptor protein subunits could act as redox–sensitive switches and thus ROS could modulate the activity of these proteins (Ciorba et al., 1997; Kolbe et al., 2010; Tang et al., 2004). For example, ryanodine receptor (RyR) and Ca^{2+} channels activity from the sarcoplasmic reticulum (SR) in the heart can be modulated by both ROS and RNS and therefore has a profound effect on the proper cardiac function and on the development of a pathophysiological condition (cardiac failure) (Zima and Blatter, 2006).

In fact ROS exert general signalling role in normal physiological processes as in many studies application of antioxidants to a system at rest or non-pathology actually attenuates the normal function of neuronal or cardiac cell excitability for example (Angelova and Muller, 2006; Wei et al., 1998)

Thus, mitochondrial ROS (and RNS), produced in this highly dependent on the redox potential milieu, are very likely to be involved in the regulation of mitochondrial ion channels and transporters (Uchi et al., 2014).

Physiological function of mitochondrial ROS – regulation of breathing

We have recently discovered a physiological role for astrocytes in regulation of breathing, appointed till recently only for the glomus cells from the carotid body (Angelova et al., 2015b). Astrocytes from the brain stem or also from the cortex were able to sense directly even slight decrease in partial pressure of atmospheric oxygen (from 21% to 15% ppO_2). Astrocytes from either primary culture, organotypic culture, acute brain slice or somatosensory cortex *in vivo* in anesthetised animal were able to respond to argon-induced hypoxia in physiological variation range with rise in cytosolic calcium under the form of sustained $[Ca^{2+}]_{i,}$ elevation, calcium oscillations or combination of both. More important, the threshold for astroglial activation of a calcium signal in the cortex lies at about 10 mmHg lower than the value for activation of the signal in glomus cells from the carotid body (37mmHg). Calcium signal evoked in astrocytes was reliably mimicked in chemical model of hypoxia (consumption of molecular oxygen by sodium dithionite (SDT) and not dependent on the activation of P2Y1 purinergic receptors, as shown for astrocytes pre-incubated with MRS2179 (a metabotropic ATP receptor antagonist).

The mitochondrion is the location where oxygen consumption ultimately takes place, for this reason we hypothesised that the sensor for astrocytic oxygen sensing resides in the mitochondria. Interestingly, in our simultaneous experiments of measurement of cytosolic calcium by fura-2 and $\Delta\psi_m$ by Rh123, each calcium spike was preceded well in advance (~30s or until mitochondrial depolarization reaches maximum drop for this condition) by a massive mitochondrial depolarization (~70%) which was reversible if episodes of hypoxia lasted no more than 2 min. Moreover, when we slightly depolarized mitochondria with 0.5 μM FCCP prior to induction of hypoxia, no more calcium signal could be evoked in astrocytes *in vitro* as well *in vivo* experiments. To double-prove this finding, we have used PINK1 (PTEN-induced kinase 1; a Parkinson's disease related gene) KO mouse astrocytes with well-established reduction of mitochondrial membrane potential as a bioenergetic consequence of the mutation and main feature of the disease (Abramov et al., 2011; Gandhi et al., 2009). As expected, no calcium signal could be evoked upon application of argon for 2 min (mild hypoxia). All this data point towards a certain threshold value for mitochondrial depolarization, that is necessary to be achieved at once, in order to trigger an astrocytic calcium response (Figure 2).

Delay in the rate of proton pumping by the enzymatic complexes of the electron transport chain will logically result in congestion of the electron transfer and thus in inhibition of mitochondrial respiration and consequently will enable the production of reactive oxygen species (Murphy, 2009) by the collision of unpaired electrons with the surrounding molecules (lipids, proteins). In (Angelova et al., 2015b), we have unravelled the mechanism of oxygen sensing of astrocytes to engage as a major step the production of mitochondrial ROS. In experiments with MitoSOX we clearly show that superoxide is produced upon induction of mild hypoxia and the rate of ROS production could be set back to basal be depolarizing the mitochondria with 0.5 μM FCCP or could be completely abolished by application of mitochondrial-targeted ROS scavenger MitoQ (Kelso et al., 2001). In fact, further step to lipid peroxidation is involved when the partial oxygen pressure $_pO_2$ was lowered, as measured in C11-BODIPY experiments in astrocytes. Again, the rate of lipid peroxidation remained at basal when astrocytes were pre-treated with 0.5 μM FCCP to slightly depolarize mitochondria prior

to the hypoxia onset. Moreover, calcium signal that has been evoked by hypoxic condition in astrocytes have been completely abolished by the application of MitoQ or Vit.E, which affirms the finding that mitochondrial ROS are able to trigger calcium signalling event in response to altered atmospheric oxygen in astrocytes (Figure 2).

Thus, we have been able to show the mechanism by which astrocytes are able to sense physiological changes in brain oxygenation and astrocytic mitochondria being the physical place of the CNS oxygen sensors.

Conclusion remarks

For long time reactive oxygen species have been considered to be by-products of biological reactions that are highly aggressive and damaging to the surrounding tissue. It is necessary to accentuate on the fact that ROS are not cellular waste products, but actually signalling molecules that are very important for proper functioning of the organism, including proliferation, differentiation, aging, transcription factor regulation, inflammation, and other regulatory functions.

There are no doubts that mitochondrial ROS are involved in the mechanism of acute (i.ie. anoxia) or chronic pathology (i.e. neurodegeneration) but because of important implication of mitochondrial ROS in physiology, more accurately developed strategy for treatment with ROS scavengers and mitochondrially targeted antioxidants should be used.

Moreover, it should also be pointed out that lipid peroxidation which has always been accepted as a marker for late oxidative stress has an essential role as a source of substrates and as an activator of phospholipases, thus playing an important role in cells housekeeping and signalling.

Acknowledgment

We thank The Leverhulme Trust for support

Figure Legend

Figure 1. Schematic representation of the major mitochondrial ROS producers and targets.

Figure 2. Mechanism of oxygen sensing in astrocytes.

Reference List

Abramov, A. Y., Gegg, M., Grunewald, A., Wood, N. W., Klein, C. and Schapira, A. H. (2011). Bioenergetic consequences of PINK1 mutations in Parkinson disease. *PLoS. One.* 6, e25622.

Abramov, A. Y., Jacobson, J., Wientjes, F., Hothersall, J., Canevari, L. and Duchen, M. R. (2005). Expression and modulation of an NADPH oxidase in mammalian astrocytes. *J. Neurosci.* 25, 9176-9184.

Abramov, A. Y., Scorziello, A. and Duchen, M. R. (2007). Three distinct mechanisms generate oxygen free radicals in neurons and contribute to cell death during anoxia and reoxygenation. *J. Neurosci.* 27, 1129-1138.

Abramov, A. Y., Smulders-Srinivasan, T. K., Kirby, D. M., Acin-Perez, R., Enriquez, J. A., Lightowlers, R. N., Duchen, M. R. and Turnbull, D. M. (2010). Mechanism of neurodegeneration of neurons with mitochondrial DNA mutations. *Brain* 133, 797-807.

Angelova, P. and Muller, W. (2006). Oxidative modulation of the transient potassium current IA by intracellular arachidonic acid in rat CA1 pyramidal neurons. *Eur. J. Neurosci.* 23, 2375-2384.

Angelova, P. R., Horrocks, M. H., Klenerman, D., Gandhi, S., Abramov, A. Y. and Shchepinov, M. S. (2015a). Lipid peroxidation is essential for alpha-synuclein-induced cell death. *J. Neurochem.* 133, 582-589.

Angelova, P. R., Kasymov, V., Christie, I., Sheikhbahaei, S., Turovsky, E., Marina, N., Korsak, A., Zwicker, J., Teschemacher, A. G., Ackland, G. L. et al. (2015b). Functional Oxygen Sensitivity of Astrocytes. *J. Neurosci.* 35, 10460-10473.

Angelova, P. R. and Muller, W. S. (2009). Arachidonic acid potently inhibits both postsynaptic-type Kv4.2 and presynaptic-type Kv1.4 IA potassium channels. *Eur. J. Neurosci.* 29, 1943-1950.

Barchas, J. D. and FREEDMAN, D. X. (1963). BRAIN AMINES: RESPONSE TO PHYSIOLOGICAL STRESS. *Biochem. Pharmacol.* 12, 1232-1235.

Boveris, A., Oshino, N. and Chance, B. (1972). The cellular production of hydrogen peroxide. *Biochem. J.* 128, 617-630.

Brand, M. D. (2010). The sites and topology of mitochondrial superoxide production. *Exp. Gerontol.* 45, 466-472.

Brennan, J. P., Southworth, R., Medina, R. A., Davidson, S. M., Duchen, M. R. and Shattock, M. J. (2006). Mitochondrial uncoupling, with low concentration FCCP, induces ROS-dependent cardioprotection independent of KATP channel activation. *Cardiovasc. Res.* 72, 313-321.

Ciorba, M. A., Heinemann, S. H., Weissbach, H., Brot, N. and Hoshi, T. (1997). Modulation of potassium channel function by methionine oxidation and reduction. *Proc. Natl. Acad. Sci. U. S. A* 94, 9932-9937.

Domijan, A. M., Kovac, S. and Abramov, A. Y. (2014). Lipid peroxidation is essential for phospholipase C activity and the inositol-trisphosphate-related Ca(2)(+) signal. *J. Cell Sci.* 127, 21-26.

Finkel, T. (2011). Signal transduction by reactive oxygen species. *J. Cell Biol.* 194, 7-15.

Fitzgerald, J. C., Ugun-Klusek, A., Allen, G., De Girolamo, L. A., Hargreaves, I., Ufer, C., Abramov, A. Y. and Billett, E. E. (2014). Monoamine oxidase-A knockdown in human neuroblastoma cells reveals protection against mitochondrial toxins. *FASEB J.* 28, 218-229.

Gandhi, S. and Abramov, A. Y. (2012). Mechanism of oxidative stress in neurodegeneration. *Oxid. Med. Cell Longev.* 2012, 428010.

Gandhi, S., Wood-Kaczmar, A., Yao, Z., Plun-Favreau, H., Deas, E., Klupsch, K., Downward, J., Latchman, D. S., Tabrizi, S. J., Wood, N. W. et al. (2009). PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol. Cell* 33, 627-638.

Gibson, G. E., Blass, J. P., Beal, M. F. and Bunik, V. (2005). The alpha-ketoglutarate-dehydrogenase complex: a mediator between mitochondria and oxidative stress in neurodegeneration. *Mol. Neurobiol.* 31, 43-63.

Gibson, G. E., Chen, H. L., Xu, H., Qiu, L., Xu, Z., Denton, T. T. and Shi, Q. (2012). Deficits in the mitochondrial enzyme alpha-ketoglutarate dehydrogenase lead to Alzheimer's disease-like calcium dysregulation. *Neurobiol. Aging* 33, 1121-1124.

Gibson, G. E., Kingsbury, A. E., Xu, H., Lindsay, J. G., Daniel, S., Foster, O. J., Lees, A. J. and Blass, J. P. (2003). Deficits in a tricarboxylic acid cycle enzyme in brains from patients with Parkinson's disease. *Neurochem. Int.* 43, 129-135.

Gonzalez-Pacheco, F. R., Caramelo, C., Castilla, M. A., Deudero, J. J., Arias, J., Yague, S., Jimenez, S., Bragado, R. and Alvarez-Arroyo, M. V. (2002). Mechanism of vascular smooth muscle cells activation by hydrogen peroxide: role of phospholipase C gamma. *Nephrol. Dial. Transplant.* 17, 392-398.

Granados, M. P., Salido, G. M., Pariente, J. A. and Gonzales, A. (2007). Modulation of CCK-8-evoked intracellular Ca2+ waves by hydrogen peroxide in mouse pancreatic acinar cells. *J. Physiol Pharmacol.* 58, 423-440.

Hare, M. L. (1928). Tyramine oxidase: A new enzyme system in liver. *Biochem. J.* 22, 968-979.

Hermann, P. M., Watson, S. N. and Wildering, W. C. (2014). Phospholipase A2 - nexus of aging, oxidative stress, neuronal excitability, and functional decline of the aging nervous system? Insights from a snail model system of neuronal aging and age-associated memory impairment. *Front Genet.* 5, 419.

Hool, L. C. and Corry, B. (2007). Redox control of calcium channels: from mechanisms to therapeutic opportunities. *Antioxid. Redox Signal.* 9, 409-435.

Kagan, V. E., Tyurin, V. A., Jiang, J., Tyurina, Y. Y., Ritov, V. B., Amoscato, A. A., Osipov, A. N., Belikova, N. A., Kapralov, A. A., Kini, V. et al. (2005). Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat. Chem. Biol.* 1, 223-232.

Kelso, G. F., Porteous, C. M., Coulter, C. V., Hughes, G., Porteous, W. K., Ledgerwood, E. C., Smith, R. A. and Murphy, M. P. (2001). Selective targeting of a redox-active ubiquinone to mitochondria within cells: antioxidant and antiapoptotic properties. *J. Biol. Chem.* 276, 4588-4596.

Kinghorn, K. J., Castillo-Quan, J. I., Bartolome, F., Angelova, P. R., Li, L., Pope, S., Cocheme, H. M., Khan, S., Asghari, S., Bhatia, K. P. et al. (2015). Loss of PLA2G6 leads to elevated mitochondrial lipid peroxidation and mitochondrial dysfunction. *Brain* 138, 1801-1816.

Kolbe, K., Schonherr, R., Gessner, G., Sahoo, N., Hoshi, T. and Heinemann, S. H. (2010). Cysteine 723 in the C-linker segment confers oxidative inhibition of hERG1 potassium channels. *J. Physiol* 588, 2999-3009.

Kovac, S., Angelova, P. R., Holmstrom, K. M., Zhang, Y., Dinkova-Kostova, A. T. and Abramov, A. Y. (2015). Nrf2 regulates ROS production by mitochondria and NADPH oxidase. *Biochim. Biophys. Acta* 1850, 794-801.

Lenaz, G. (2001). The mitochondrial production of reactive oxygen species: mechanisms and implications in human pathology. *IUBMB. Life* 52, 159-164.

Liu, Y. and Gutterman, D. D. (2002). Oxidative stress and potassium channel function. *Clin. Exp. Pharmacol. Physiol* 29, 305-311.

Loschen, G., Azzi, A., Richter, C. and Flohe, L. (1974). Superoxide radicals as precursors of mitochondrial hydrogen peroxide. *FEBS Lett.* 42, 68-72.

Loschen, G., Flohe, L. and Chance, B. (1971). Respiratory chain linked H(2)O(2) production in pigeon heart mitochondria. *FEBS Lett.* 18, 261-264.

Marzoev, A., Mirtalipov, D. and Almatov, K. (1987). [Role of mitochondrial peroxidation of lipids in their hydrolysis by endogenous phospholipase A2]. *Biull. Eksp. Biol. Med.* 104, 35-38.

Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochem. J.* 417, 1-13.

Natarajan, V. and Garcia, J. G. (1993). Agonist-induced activation of phospholipase D in bovine pulmonary artery endothelial cells: regulation by protein kinase C and calcium. *J. Lab Clin. Med.* 121, 337-347.

Nohl, H., Koltover, V. and Stolze, K. (1993). Ischemia/reperfusion impairs mitochondrial energy conservation and triggers O2.- release as a byproduct of respiration. *Free Radic. Res. Commun.* 18, 127-137.

Rossi, M. A., Di, M. C., Esterbauer, H., Fidale, F. and Dianzani, M. U. (1994). Activation of phosphoinositide-specific phospholipase C of rat neutrophils by the chemotactic aldehydes 4-hydroxy-2,3-trans-nonenal and 4-hydroxy-2,3-trans-octenal. *Cell Biochem. Funct.* 12, 275-280.

Shi, Q., Xu, H., Yu, H., Zhang, N., Ye, Y., Estevez, A. G., Deng, H. and Gibson, G. E. (2011). Inactivation and reactivation of the mitochondrial alpha-ketoglutarate dehydrogenase complex. *J. Biol. Chem.* 286, 17640-17648.

Skulachev, V. P. (1996). Role of uncoupled and non-coupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants. *Q. Rev. Biophys.* 29, 169-202.

Starkov, A. A. and Fiskum, G. (2003). Regulation of brain mitochondrial H2O2 production by membrane potential and NAD(P)H redox state. *J. Neurochem.* 86, 1101-1107.

Starkov, A. A., Fiskum, G., Chinopoulos, C., Lorenzo, B. J., Browne, S. E., Patel, M. S. and Beal, M. F. (2004). Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. *J. Neurosci.* 24, 7779-7788.

Tang, X. D., Daggett, H., Hanner, M., Garcia, M. L., McManus, O. B., Brot, N., Weissbach, H., Heinemann, S. H. and Hoshi, T. (2001). Oxidative regulation of large conductance calcium-activated potassium channels. *J. Gen. Physiol* 117, 253-274.

Tang, X. D., Garcia, M. L., Heinemann, S. H. and Hoshi, T. (2004). Reactive oxygen species impair Slo1 BK channel function by altering cysteine-mediated calcium sensing. *Nat. Struct. Mol. Biol.* 11, 171-178.

Timusheva, Y. T., Mareninova, O. A., Vagina, O. N., Zamaraeva, M. V., Salakhutdinov, B. A., Aripov, T. F. and Tashmukhamedov, B. A. (1998). The role of membrane structure in the activation of mitochondrial phospholipases. 1. Activation of mitochondrial phospholipases by lipid peroxidation products. *Membr. Cell Biol.* 12, 41-49.

Tretter, L. and Adam-Vizi, V. (2000). Inhibition of Krebs cycle enzymes by hydrogen peroxide: A key role of [alpha]-ketoglutarate dehydrogenase in limiting NADH production under oxidative stress. *J. Neurosci.* 20, 8972-8979.

Tretter, L. and Adam-Vizi, V. (2004). Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. *J. Neurosci.* 24, 7771-7778.

Uchi, J., Ryu, S. Y., Jhun, B. S., Hurst, S. and Sheu, S. S. (2014). Mitochondrial ion channels/transporters as sensors and regulators of cellular redox signaling. *Antioxid. Redox Signal.* 21, 987-1006.

Vaarmann, A., Gandhi, S. and Abramov, A. Y. (2010). Dopamine induces Ca2+ signaling in astrocytes through reactive oxygen species generated by monoamine oxidase. *J. Biol. Chem.* 285, 25018-25023.

van Rossum, G. S., Drummen, G. P., Verkleij, A. J., Post, J. A. and Boonstra, J. (2004). Activation of cytosolic phospholipase A2 in Her14 fibroblasts by hydrogen peroxide: a p42/44(MAPK)-dependent and phosphorylation-independent mechanism. *Biochim. Biophys. Acta* 1636, 183-195.

Votyakova, T. V. and Reynolds, I. J. (2001). DeltaPsi(m)-Dependent and -independent production of reactive oxygen species by rat brain mitochondria. *J. Neurochem.* 79, 266-277.

Wei, E. P., Kontos, H. A. and Beckman, J. S. (1998). Antioxidants inhibit ATP-sensitive potassium channels in cerebral arterioles. *Stroke* 29, 817-822.

Zhang, L., Yu, L. and Yu, C. A. (1998). Generation of superoxide anion by succinate-cytochrome c reductase from bovine heart mitochondria. *J. Biol. Chem.* 273, 33972-33976.

Zima, A. V. and Blatter, L. A. (2006). Redox regulation of cardiac calcium channels and transporters. *Cardiovasc. Res.* 71, 310-321.

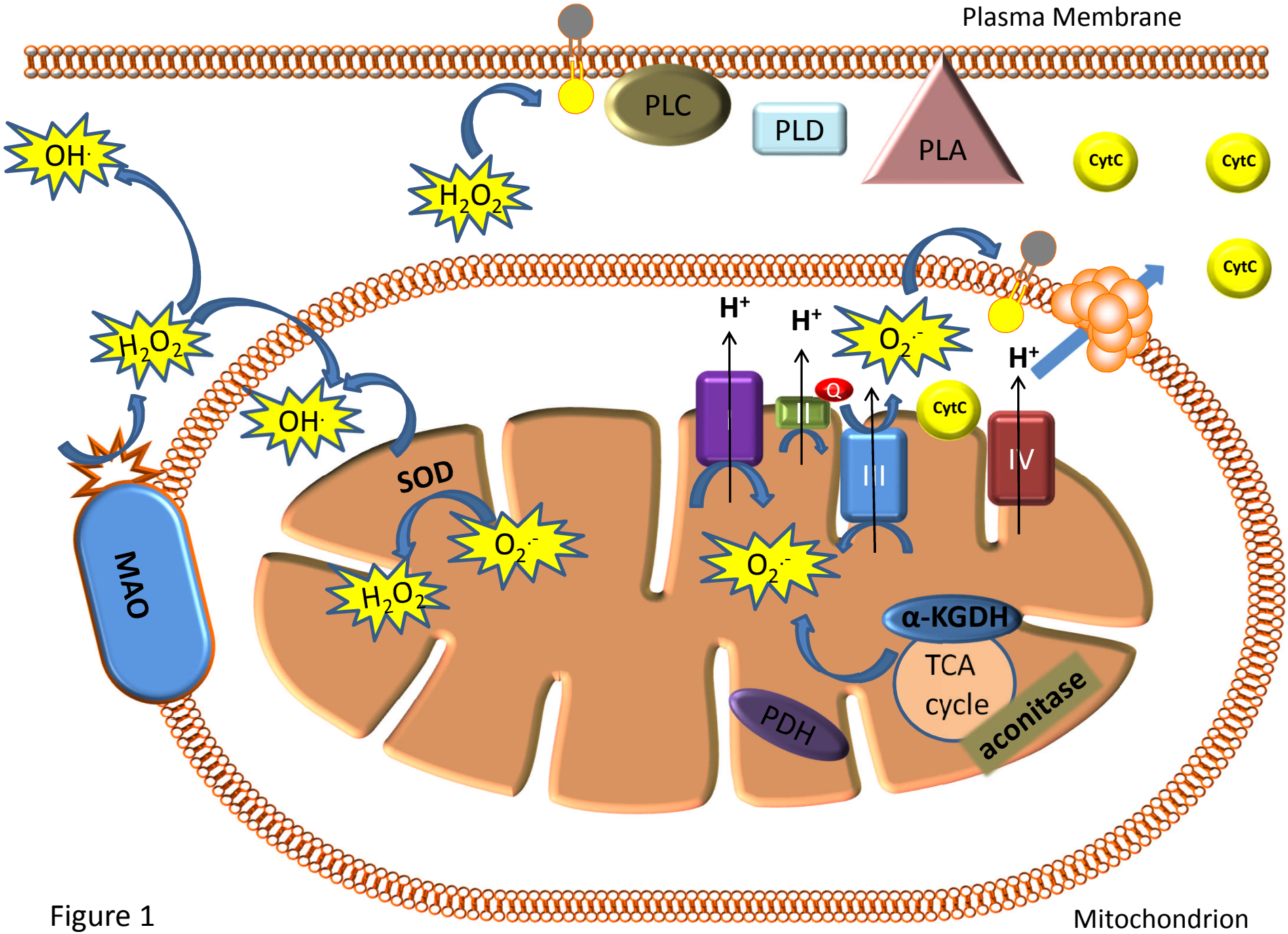
Plasma Membrane
PLC
PLD
PLA
CytC
CytC
CytC
OH·
H_2O_2
H_2O_2
OH·
MAO
SOD
$O_2^{\cdot -}$
H_2O_2
$O_2^{\cdot -}$
H+
H+
H+
Q
I
II
III
IV
CytC
α-KGDH
TCA
cycle
aconitase
PDH
Mitochondrion

Figure 1

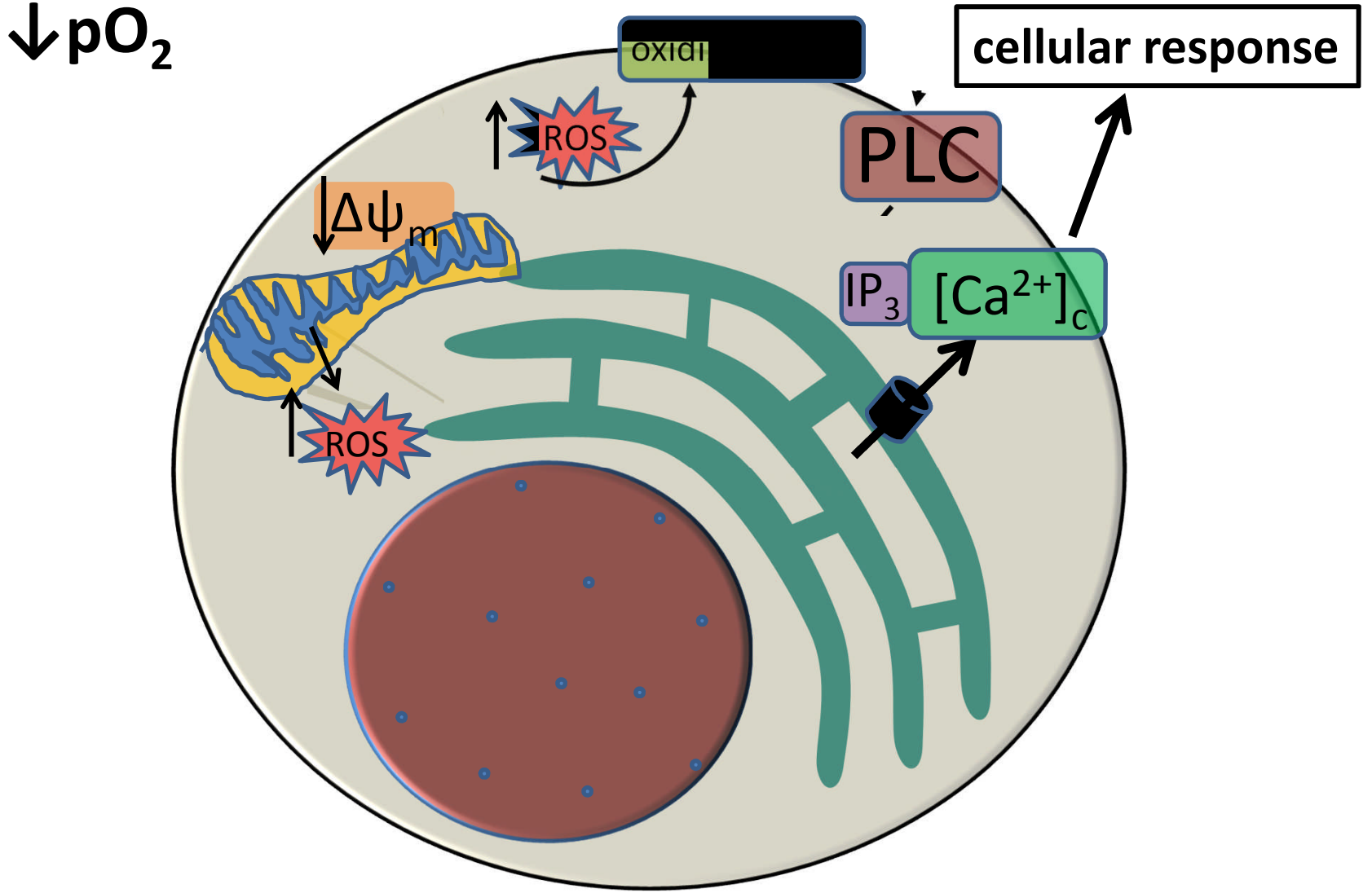

Figure 2