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Guanosine diphosphate exerts a lower effect on superoxide release from mitochondrial matrix in the brains of uncoupling protein-2 knockout mice: New evidence for a putative novel function of uncoupling proteins as superoxide anion transporters

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ABSTRACT

In this report, we show new experimental evidence that, in mouse brain mitochondria, uncoupling protein-2 (UCP2) can be involved in superoxide ($O_2^{\cdot -}$) removal from the mitochondrial matrix. We found that the effect of guanosine 5′-diphosphate (GDP) on the rate of reactive oxygen species (ROS) release from brain mitochondria of UCP2 knockout mice was less pronounced compared to the wild type animals. This putative novel UCP2 activity, evaluated by the use of UCP2-knockout transgenic animals, along with the known antioxidant defence systems, may provide additional protection from ROS in brain mitochondria.

1. Introduction

Traditionally, uncoupling protein 1 (UCP1) has been associated with thermogenesis in brown adipose tissue in which it is involved in the production of heat as a consequence of the dissipation of the mitochondrial proton gradient in response to noradrenergic stimulation (for a review, see [1]). However, the uncoupling activity of the subsequently discovered UCP2, UCP3 [2,3], UCP4 and BMCP1/UCP5 [4,5] observed by several other authors (e.g., [6,7]) has not been fully understood and raises many unanswered questions, leading to often contradictory conclusions (for reviews, see [8–10]). Nevertheless, UCPs are involved in decreasing the production of ROS [11,12], and thus they are important players in a number of pathologies, such as obesity, diabetes [13,14], cancer [15,16] and neurological disorders [17–21]. Our earlier studies [22] indicated for the first time that the UCPs from rat heart and skeletal muscle might also play an additional role as superoxide carriers. This hypothesis was initially confirmed by experiments using mitochondria isolated only from wild-type animals. In this paradigm, the UCPs contribute to the transport of superoxide from the matrix to the intermembrane space (IMS). The main objective of this study was to evaluate this hypothesis with the use of transgenic UCP2-knockout mice and expand the hypothesis to UCP2 in mouse brain mitochondria.

2. Materials and methods

All of the experiments were performed in accordance with the guidelines approved by the Local Ethical Committee based on national laws that are in full agreement with the European Union directive on animal experimentation.

2.1. Animals

Swiss male mice were used for the initial experiments on wild-type animals. The UCP2 KO experiments were performed using C57Bl/6 male mice lacking UCP2 and their wild type controls aged 10–12 weeks were derived from those described in [23]. The UCP2 KO mice used in this study were backcrossed for more than 10 generations onto a C57Bl/6 background.

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2.2. Materials

Amplex Red was obtained from Invitrogen (USA). The other chemicals and enzymes were purchased from Sigma–Aldrich (Germany, Poland and Sweden), BioRad (Poland) and Roche (Sweden).

2.3. Preparation of brain mitochondria fraction

For each preparation, the brains from two mice were removed, sliced and washed in isolation medium containing 75 mM sucrose, 225 mM mannitol, 25 mM Tris–HCl and 0.5 mM EGTA, pH 7.4, at 4 °C. The brains were then homogenised in 10 ml per g of tissue of the same medium and centrifuged for 5 min at 1330*g*. The pellet was discarded, and the supernatant was centrifuged at 8000*g* for 10 min. Subsequently, the supernatant was discarded, and the pellet was resuspended in the above isolation medium and centrifuged for 10 min at 9000*g*. The obtained pellet was resuspended again in the same medium and centrifuged for 10 min at 9000*g*. Lastly, the pellet was resuspended in approx. 1 ml of the isolation medium.

2.4. Protein quantification

The protein concentration in the mitochondrial fraction was determined according to Bradford's method using a Bio-Rad Protein Assay (BioRad) or Fluram (Roche).

2.5. Incubations

The standard incubation medium was composed of either 225 mM mannitol, 75 mM sucrose, 25 mM Tris–HCl and 0.5 mM EGTA, pH 7.4 (mannitol/sucrose medium), or 110 mM mannitol, 60 mM KCl, 60 mM Tris–HCl, 10 mM KH_2PO_4 and 0.5 mM EDTA, pH 7.4 (mannitol/KCl medium).

2.6. ROS measurements

The total release of ROS from the mitochondria to the external medium was estimated fluorometrically by the oxidation of Amplex Red (non-fluorescent) to resorufin (fluorescent). Briefly, the mitochondria (0.2 mg protein/ml) were incubated at 26 °C in the standard incubation medium supplemented with 5 mM succinate, 5 μM Amplex Red plus Cu/Zn-superoxide dismutase (2 U/ml) and horseradish peroxidase (2 U/ml). The fluorescence of resorufin, the oxidation product of Amplex Red, was measured using a Sigma spectrofluorometer (Germany). The increase in the fluorescence at the excitation wavelength of 545 nm and emission via a band pass filter of 600 ± 20 nm was followed using a 3 ml cuvette. The response of the resorufin fluorescence was calibrated with H_2O_2. Alternatively, the fluorescence was measured using a multiwell plate reader (Infinite M200, Tecan, Austria) at 26 °C with 510 ± 10 nm excitation and 595 ± 35 nm emission wavelengths in 24 well plates.

2.7. Aconitase measurements

The release of $O_2^{\cdot-}$ into the mitochondrial matrix compartment was estimated by measuring the degree of inactivation of the mitochondrial enzyme aconitase [22,24] by recording the rate of $NADP^+$ reduction, as described previously [25]. Briefly, the standard incubation medium was supplemented with 5 mM glutamate, 5 mM malate, 5 mM citrate, 0.8 mM $NADP^+$, 0.6 mM $MnCl_2$ and isocitrate dehydrogenase (2 U/ml), followed by 0.05% (v/v) lauroyl maltoside to enable the access of the reagents to the matrix compartment. The rate of NADPH formation was recorded spectrophotometrically at 340 nm and 37 °C for 10 min.

2.8. Data analysis

The data are reported as the means ± SD, and the results are presented as a percentage of the control group (control value = 100%). The statistical significance was estimated using Student's *t*-test for the results presented as the percentage of the control. A value of $p \leqslant 0.05$ was assumed to be statistically significant and is marked with an asterisk (∗).

3. Results

Similarly to skeletal muscle and heart mitochondria studied in our previous work [22], mouse brain mitochondria were subjected to conditions of increased ROS production. Isolated mitochondria were treated with antimycin A (an inhibitor of complex III) to reach a vast increase in ROS and the collapse of mitochondrial membrane potential [26]. We observed that, under such conditions, the release of ROS from the mouse brain mitochondria, measured as H_2O_2 in the external medium, was substantially decreased by the addition of 0.5 mM GDP (Fig. 1A). Similar inhibitory effects were observed for GTP. Because guanosine phosphates at concentrations higher than 1 mM may interfere with the Amplex Red assay (see also [27]), the GDP and GTP used in these experiments were kept at a level of 0.5 mM, a concentration that exerted no or negligible effects on the assay (data not shown). The addition of albumin (BSA) increased the ROS release due to the elimination of endogenous free fatty acids (FFAs), which could potentially compete with $O_2^{\cdot-}$ for the UCP transport function. The rate of $O_2^{\cdot-}$ release from the mitochondrial matrix was increased when the FFAs were bound by BSA. In contrast, the addition of myristate (125 nmol/mg protein) decreased the release of ROS (Fig. 1B).

The reduced release of ROS in the presence of guanine di- and trinucleotides may result from either their decreased formation inside the mitochondria or decreased export across the inner membrane to the external medium. To resolve this issue, the activity of aconitase, an enzyme present in the matrix compartment and involved in the tricarboxylic acid cycle that is extremely sensitive to ROS due to the presence of a labile iron–sulphur cluster [24] was measured. Therefore, its level may be used to reflect the level of ROS inside the mitochondria. It appeared (Table 1) that the presence of 1 mM GTP resulted in a decrease in aconitase activity, clearly indicating an increase in the ROS concentration in the matrix compartment.

To evaluate the participation of UCP2 in $O_2^{\cdot-}$ release, transgenic mice lacking the UCP2 protein were used. As presented in Fig. 2, the effect of GDP was smaller in the UCP2-knockout animals compared to their wild-type counterparts. Cytidine 5-diphosphate (CDP), a pyrimidine nucleotide with no affinity for UCP, demonstrated no effect on the ROS release in either genotype.

It has been repeatedly demonstrated that the mitochondria isolated from the tissues and organs of old animals produce more ROS than those of younger subjects. To examine a possible adaptation to the increased ROS production, the age-dependent differences in the UCP-mediated ROS release were evaluated. The effect of GDP was assessed in 2- and 24-month-old wild-type mice (Fig. 3A) and accounted for a 15% ± 3% decrease in the ROS released from the antimycin A-inhibited brain mitochondria of younger animals compared to a 28% ± 1% decrease in the older subjects. The level of the UCP2 protein was comparable in the young and old individuals.

4. Discussion

Traditionally, the relationship between mitochondrially generated ROS and UCP activity has been considered in terms of either the $O_2^{\cdot-}$-induced activation of the protonophoric activity of UCP or the alleviation of ROS release from the mitochondria [28,29].

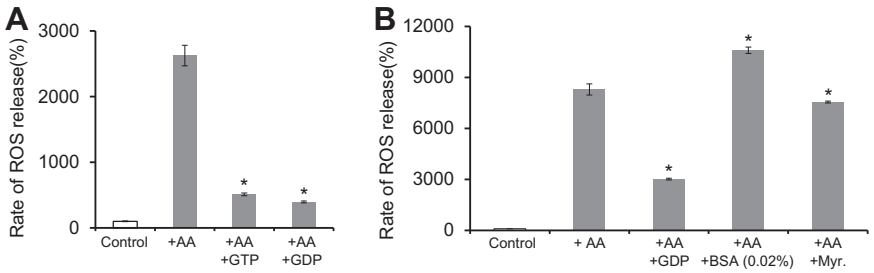

Fig. 1. Effect of GDP on the ROS release from mouse brain mitochondria. (A) Effect of GDP on ROS release from non-respiring and ROS-overproducing mitochondria. (B) Effect of free fatty acids on the ROS release from non-respiring and ROS-overproducing mitochondria. The ROS release and oxygen consumption were measured as described in Section 2 in the presence of 200 μg mitochondrial protein. Other additions include 1 μM antimycin A (AA) and 0.5 mM GDP. The data shown are the means ± SD, $^{*}p < 0.05$ ($n = 4$) versus AA (Fig. 1A) for each parameter.

Table 1
Effect of GTP on aconitase activity in respiring mouse brain mitochondria. Aconitase was measured as described in Section 2 in the absence (control) or presence of 1 mM GTP. The data shown are the means ± SD, $^{*}p < 0.05$ ($n = 3$).

	Aconitase activity	
	(nmol NADPH/min/mg protien)	(% of control)
Control	86.3 ± 1.2	100
+1 mM GTP	76.3 ± 6.1	88*

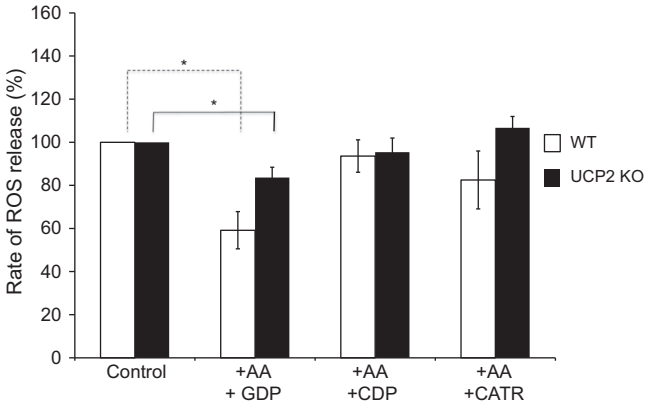

Fig. 2. Effect of GDP on the ROS release from control and UCP2-knockout mouse brain mitochondria. The conditions and additions are as in Fig. 1. Other additions include 0.5 mM CDP and 1 μM CATR. The data shown are the means ± SD, $^{*}p < 0.05$ ($n = 4$) versus the respective controls.

Moreover, UCP-mediated mild uncoupling may regulate ROS production via controlling the mitochondrial coupling [30]. However, these classic views are constantly being challenged and re-evaluated because of new findings in what has emerged to be a stimulating discussion, with great benefit to the topic [8–10]. We previously demonstrated that UCP3 in heart and muscle mitochondria may mediate the transport of matrix $O_2^{\cdot -}$ across the inner mitochondrial membrane [22]. The data presented in this paper show that mouse brain mitochondrial UCP2 may also be involved in this process.

In isolated mitochondria, the electron transfer using succinate as the respiratory substrate leads to the overproduction of ROS due to the non-physiological concentration of the substrate, which is much higher than usually present in mitochondria of intact cells [8]. Additionally, antimycin A, an inhibitor of complex III, causes a further substantial increase in $O_2^{\cdot -}$ generation, particularly from the Qo site, and its release to both sides of the inner membrane. The $O_2^{\cdot -}$ generated by leaks at the Qi site predominantly occurs on the matrix side [31]. In the present study, non-respiring mitochondria producing high amounts of ROS were used to estimate the role of UCP2 in the transport of superoxide (Fig. 1A).

Because $O_2^{\cdot -}$ cannot penetrate the inner membrane, the only means of protecting the mitochondrial interior against this harmful molecule would be either to convert it immediately to H_2O_2 for further processing or export it from this compartment. The former activity is catalysed by SOD2 within the matrix, and the H_2O_2 produced in this process is able to pass freely through the membranes and may play a pivotal role in the progression of oxidative stress or cellular signalling [32]. The remaining $O_2^{\cdot -}$ has been proposed to be exported by UCP isoforms, a process inhibited by purine nucleotides (Fig. 1A). Long-chain FFAs, are believed to be transported by different mitochondrial carriers, such as adenine nucleotide trans-

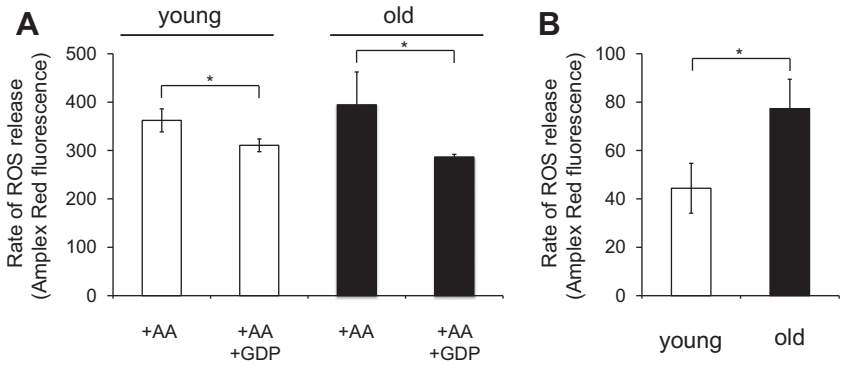

Fig. 3. Age-dependent effect of GDP on the ROS release from the mouse brain mitochondria isolated from young and old animals. (A) Effect of GDP on ROS release from AA treated mitochondria. (B) Basal (non AA-induced) ROS release. The conditions and additions are as in Fig. 1. The data shown are the means ± SD, $^{*}p < 0.05$ ($n = 4$).

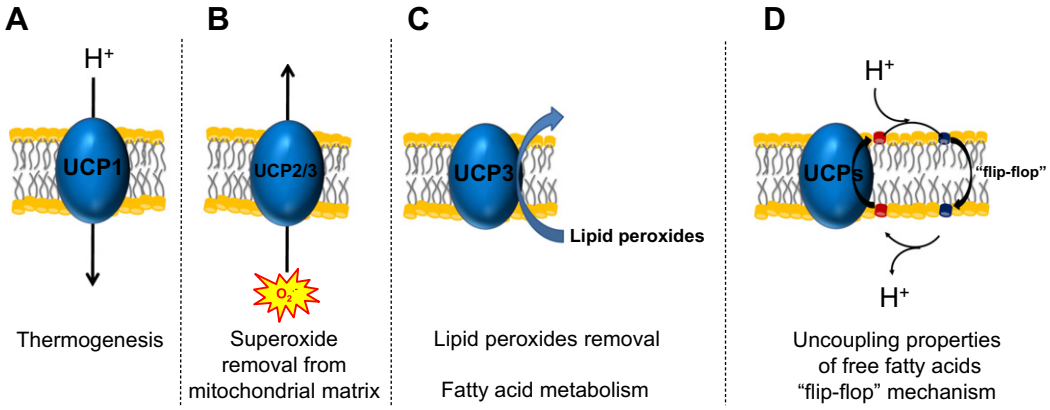

Fig. 4. The "traditional" and "novel" functions of UCP proteins.

locase (ANT), dicarboxylate and glutamate/aspartate carriers, a phosphate carrier [33] and UCPs [34], and participate in the uncoupling of oxidative phosphorylation. Therefore, FFAs might be engaged in potential competition with $O_2^{\cdot-}$ for the channelling activity of the UCP protein, as demonstrated by the experiment in which the addition of an FFA-binding agent, albumin, boosted the detected amount of ROS released from the mitochondria (Fig. 1B). Moreover, we observed that the addition of myristate decreased the release of ROS from the brain mitochondria (Fig. 1B). Because these are non-respiring mitochondria, the myristate effect cannot be considered as a mild uncoupling and, thus, excludes the FFA-uncoupling activity in our experimental model. This result further supports the idea that UCP2 can be involved in superoxide removal from the mitochondrial matrix. Moreover, we used transgenic animals to assess the significance of UCP2 in this process. The effect of GDP in the mitochondria from the wild-type mice was much stronger compared to when the UCP2-knockout animals were used (Fig. 2). Because the brain contains UCP2 and also UCP4 and UCP5 [4,5], the potential involvement of these proteins in this process should also be considered. The particular structures of purine nucleoside tri- and di-phosphates (but not purine nucleoside monophosphates) allow their binding with high affinity to the nucleotide-binding site of UCP [35]. Therefore, the pyrimidine nucleosides (e.g., cytidine diphosphate, CDP), which do not share this structure, do not exhibit such inhibitory activity (Fig. 2). In contrast, the involvement of other mitochondrial carrier proteins may be considered because carboxyatractyloside (CATR), an inhibitor of ANT, also demonstrated a tendency, though insignificant, toward an inhibitory effect on ROS release in the wild-type mice. This result may point to the potential contribution of ANT to the studied phenomenon. This tendency should be elucidated in further studies.

Furthermore, our findings were based on the observations that GTP and GDP decreased the release of ROS from non-respiring mitochondria when measured extramitochondrially (Amplex Red/HRP). Additionally, based on the aconitase assay, we observed that these nucleotides increased the intramitochondrial accumulation of ROS, which pointed to the inhibition of transport rather than the inhibition of generation (Table 1) [28].

Based on our current results, it is tempting to recall our previous hypothesis that, along with the 'traditional' mechanism of UCP-mediated oxidative stress prevention (by limiting ROS production), UCP can play a dual function in preventing ROS toxicity. The pivotal effect of the GDP activity on the ROS production observed by other authors [27,36] could be due to the prevalence of the recoupling activity of GDP rather than the proposed $O_2^{\cdot-}$ transport. Such variability may also be due to different experimental procedures, resulting in diverse extents of ubiquinone reduction and influencing the effect of GDP on the UCP proteins [37,38].

Therefore, by evaluating our hypothesis with experiments using transgenic animals, we are closer to elucidating the mechanism involved. Although we should re-consider the importance of other mitochondrial carrier proteins, the involvement of UCP2 as a transporter of $O_2^{\cdot-}$ seems likely.

Interestingly, the effect of GDP was stronger in the mitochondria isolated from the 2-year-old mice compared to the 2-month-old subjects (28 vs. 15%, respectively) (Fig. 3A), even though the level of UCP2 was comparable (data not shown). This fact may point to an increased involvement of UCP, indicating an increased adaptation to the higher ROS production in the older individuals (Fig. 3B).

Moreover, in order to verify our hypothesis further, a study using UCP3-knockout animals is being conducted, and experiments with UCP overexpression are being planned.

Revealing the cellular pathways associated with ROS management is of particular importance from both the point of view of basic science and because of potential implications for medicine. From this work, we conclude that, along with the roles of superoxide dismutases, glutathione peroxidase and other antioxidant systems, UCP2 may constitute an important component of the machinery protecting mitochondria in the brain and other tissues against the damaging effects of oxygen free radicals. The "traditional" and "novel" functions of UCPs are summarised in Fig. 4.

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References

[1] B. Cannon, J. Nedergaard, Brown adipose tissue: function and physiological significance, Physiol. Rev. 84 (2004) 277–359.
[2] C. Fleury, M. Neverova, S. Collins, et al., Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia, Nat. Genet. 15 (1997) 269–272.
[3] O. Boss, S. Samec, A. Paoloni-Giacobino, et al., Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression, FEBS Lett. 408 (1997) 39–42.
[4] W. Mao, X.X. Yu, A. Zhong, et al., A novel brain-specific mitochondrial protein that reduces membrane potential in mammalian cells, FEBS Lett. 443 (1999) 326–330.
[5] D. Sanchis, C. Fleury, N. Chomiki, et al., BMCP1, a novel mitochondrial carrier with high expression in the central nervous system of humans and rodents, and respiration uncoupling activity in recombinant yeast, J. Biol. Chem. 273 (1998) 34611–34615.
[6] U. De Marchi, C. Castelbou, N. Demaurex, Uncoupling protein 3 (UCP3) modulates the activity of Sarco/endoplasmic reticulum Ca^{2+}ATPase (SERCA) by decreasing mitochondrial ATP production, J. Biol. Chem. 286 (2011) 32533–32541.
[7] K.S. Echtay, E. Winkler, K. Frischmuth, M. Klingenberg, Uncoupling proteins 2 and 3 are highly active H(+) transporters and highly nucleotide sensitive when activated by coenzyme Q (ubiquinone), Proc. Natl. Acad. Sci. USA 98 (2001) 1416–1421.
[8] I.G. Shabalina, J. Nedergaard, Mitochondrial ('mild') uncoupling and ROS production: physiologically relevant or not?, Biochem Soc. Trans. 39 (2011) 1305–1309.
[9] V. Azzu, M. Jastroch, A.S. Divakaruni, M.D. Brand, The regulation and turnover of mitochondrial uncoupling proteins, Biochim. Biophys. Acta 1797 (2010) 785–791.
[10] V. Azzu, M.D. Brand, The on-off switches of the mitochondrial uncoupling proteins, Trends Biochem. Sci. 35 (2010) 298–307.
[11] R.J. Mailloux, M.E. Harper, Uncoupling proteins and the control of mitochondrial reactive oxygen species production, Free Radic. Biol. Med. 51 (2011) 1106–1115.
[12] C. Duval, A. Negre-Salvayre, A. Dogilo, et al., Increased reactive oxygen species production with antisense oligonucleotides directed against uncoupling protein 2 in murine endothelial cells, Biochem. Cell Biol. 80 (2002) 757–764.
[13] J. Diao, E.M. Allister, V. Koshkin, et al., UCP2 is highly expressed in pancreatic alpha-cells and influences secretion and survival, Proc. Natl. Acad. Sci. USA 105 (2008) 12057–12062.
[14] C.Y. Zhang, G. Baffy, P. Perret, et al., Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, beta cell dysfunction, and type 2 diabetes, Cell 105 (2001) 745–755.
[15] I. Samudio, M. Fiegl, M. Andreeff, Mitochondrial uncoupling and the Warburg effect: molecular basis for the reprogramming of cancer cell metabolism, Cancer Res. 69 (2009) 2163–2166.
[16] M.E. Harper, A. Antoniou, E. Villalobos-Menuey, et al., Characterization of a novel metabolic strategy used by drug-resistant tumor cells, FASEB J.: Off. Publ. Fed. Am. Soc. Exp. Biol. 16 (2002) 1550–1557.
[17] A.D. Gigante, A.C. Andreazza, B. Lafer, et al., Decreased mRNA expression of uncoupling protein 2, a mitochondrial proton transporter, in post-mortem prefrontal cortex from patients with bipolar disorder and schizophrenia, Neurosci. Lett. 505 (2011) 47–51.
[18] T. Deierborg, T. Wieloch, S. Diano, et al., Overexpression of UCP2 protects thalamic neurons following global ischemia in the mouse, J. Cereb. Blood Flow Metab.: Official J. Int. Soc. Cereb. Blood Flow Metab. 28 (2008) 1186–1195.
[19] S. Diano, R.T. Matthews, P. Patrylo, et al., Uncoupling protein 2 prevents neuronal death including that occurring during seizures: a mechanism for preconditioning, Endocrinology 144 (2003) 5014–5021.
[20] G. Mattiasson, M. Shamloo, G. Gido, et al., Uncoupling protein-2 prevents neuronal death and diminishes brain dysfunction after stroke and brain trauma, Nat. Med. 9 (2003) 1062–1068.
[21] P.G. Sullivan, C. Dube, K. Dorenbos, et al., Mitochondrial uncoupling protein-2 protects the immature brain from excitotoxic neuronal death, Ann. Neurol. 53 (2003) 711–717.
[22] L. Wojtczak, M. Lebiedzinska, J.M. Suski, et al., Inhibition by purine nucleotides of the release of reactive oxygen species from muscle mitochondria: indication for a function of uncoupling proteins as superoxide anion transporters, Biochem. Biophys. Res. Commun. 407 (2011) 772–776.
[23] D. Arsenijevic, H. Onuma, C. Pecqueur, et al., Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production, Nat. Genet. 26 (2000) 435–439.
[24] P.R. Gardner, Aconitase: sensitive target and measure of superoxide, Methods Enzymol. 349 (2002) 9–23.
[25] D.A. Talbot, M.D. Brand, Uncoupling protein 3 protects aconitase against inactivation in isolated skeletal muscle mitochondria, Biochim. Biophys. Acta 1709 (2005) 150–156.
[26] M.P. Murphy, How mitochondria produce reactive oxygen species, Biochem. J. 417 (2009) 1–13.
[27] L.J. Toime, M.D. Brand, Uncoupling protein-3 lowers reactive oxygen species production in isolated mitochondria, Free Radic. Biol. Med. 49 (2010) 606–611.
[28] K.S. Echtay, Mitochondrial uncoupling proteins–what is their physiological role?, Free Rad Biol. Med. 43 (2007) 1351–1371.
[29] V. Bezaire, E.L. Seifert, M.E. Harper, Uncoupling protein-3: clues in an ongoing mitochondrial mystery, FASEB J.: Off. Publ. Fed. Am. Soc. Exp. Biol. 21 (2007) 312–324.
[30] S.S. Korshunov, V.P. Skulachev, A.A. Starkov, High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria, FEBS Lett. 416 (1997) 15–18.
[31] F.L. Muller, Y. Liu, H. Van Remmen, Complex III releases superoxide to both sides of the inner mitochondrial membrane, J. Biol. Chem. 279 (2004) 49064–49073.
[32] B. D'Autreaux, M.B. Toledano, ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis, Nat. Rev. Mol. Cell Biol. 8 (2007) 813–824.
[33] L. Wojtczak, M.R. Wieckowski, The mechanisms of fatty acid-induced proton permeability of the inner mitochondrial membrane, J. Bioenerg. Biomembr. 31 (1999) 447–455.
[34] V.P. Skulachev, Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation, FEBS Lett. 294 (1991) 158–162.
[35] T. Hagen, C.Y. Zhang, C.R. Vianna, B.B. Lowell, Uncoupling proteins 1 and 3 are regulated differently, Biochemistry 39 (2000) 5845–5851.
[36] A. Dlaskova, K.J. Clarke, R.K. Porter, The role of UCP 1 in production of reactive oxygen species by mitochondria isolated from brown adipose tissue, Biochim. Biophys. Acta 1797 (2010) 1470–1476.
[37] A. Swida-Barteczka, A. Woyda-Ploszczyca, F.E. Sluse, W. Jarmuszkiewicz, Uncoupling protein 1 inhibition by purine nucleotides is under the control of the endogenous ubiquinone redox state, Biochem. J. 424 (2009) 297–306.
[38] A. Swida, A. Woyda-Ploszczyca, W. Jarmuszkiewicz, Redox state of quinone affects sensitivity of Acanthamoeba castellanii mitochondrial uncoupling protein to purine nucleotides, Biochem. J. 413 (2008) 359–367.