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Review

UCP2, not a physiologically relevant uncoupler but a glucose sparing switch impacting ROS production and glucose sensing

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ABSTRACT

In mammals the two proteins UCP2 and UCP3 are highly similar to the mitochondrial uncoupling protein found in the brown adipose tissue (UCP1). Accordingly, it was proposed that UCP2 and UCP3 are also uncoupling proteins *i.e.* protonophores with impact on mitochondrial ROS production and glucose signaling. However, it appears now impossible to explain the physiological relevance of the new UCPs uniquely by their uncoupling activity as observed *in vitro*. Therefore, we propose a metabolic hypothesis in which UCP2 acts through a transport distinct of the proton transport. A consequence of this transport activity would be a decrease of the mitochondrial oxidation of the pyruvate originating from glucose. This would put UCP2 and UCP3 in a crucial position to influence cellular metabolism. The tight control exerted on UCP2 expression appears consistent with it. In this hypothesis, UCP2/3 would allow a cell to remain glycolytic within an aerobic organism. This tallies with the high expression level of UCP2 or UCP3 in glycolytic cells. The metabolic hypothesis would explain the spectacular modifications associated with UCP2 manipulation as well as the uncoupling activity usually called for and which in fact remains elusive *in vivo*.

1. Introduction

An increasing number of reports point to a relevance of the UCP2 for important physiological functions/events: data obtained with mice in which UCP2 expressed has been knocked-in or knock-down, have shown the relevance of UCP2 for the control of immune cell function [1], cellular response to glucose and therefore insulin release [2,3]. These observations deal with normal physiology where in some cases the biological fitness of *Ucp2-KO* mice appeared better [1,2]. Therefore, the presence of UCP2 has both beneficial or adverse [4] effects depending on the situation. In other studies dealing with survival of cells/organs to shocks (ischemia–reperfusion, trauma) the value of UCP2 (or other UCPs, see below) appeared generally positive with remarkable cases of protection as shown for example with UCP2 in the central nervous system [5]. As UCP2 is a mitochondrial protein, these observations have to be related to changes in mitochondrial activity linked to the presence/absence of UCP2.

2. Uncoupling the mitochondrial respiration

UCP is the acronym for UnCoupling Protein [6], this acronym was introduced for the mitochondrial uncoupling protein (now UCP1) found in the brown adipose tissue of mammals. UCP1 was the first uncoupling protein discovered in 1976. After 1996 new UCPs were discovered: UCP2 and UCP3 in mammals, and related proteins in other phyla including plants [7]. Concerning mitochondrial activity, uncoupling refers to a situation in which the mitochondrial respiration is made less (no more) dependent upon the phosphorylation of ADP into ATP. According to the Mitchell's chemiosmotic theory the simplest way to achieve uncoupling would be the introduction of a passive proton conductance in the mitochondrial inner membrane. This is clearly what UCP1 does in the brown adipocyte. The actual mechanism involved is still a matter of controversies [8,9]. Whatever the mitochondrial uncoupling mechanism would be, it has several consequences at the mitochondrial/cellular level: 1) it decreases mitochondrial membrane potential; 2) it accelerates mitochondrial respiration; 3) it diminishes the ATP/ADP ratio; 4) it results in a more oxidized state of the cells (ratio NADH/NAD decreases); 5) it reduces the mitochondrial production of reactive oxygen species. However, the observation of one (or more) of these consequences in a cell is not an absolute proof of uncoupling as for example: an acceleration of ATP consumption would produce the same modifications.

The use of oligomycin provides a possibility to distinguish uncoupling from increased ATP turnover. Oligomycin inhibits the mitochondrial ATPsynthase. Hence, when normal mitochodrial bioenergetics takes place (respiratory chain creates a proton gradient that is used to phosphorylate ADP into ATP) oligomycin reduces cellular respiration (often by a factor 3 or 4) and increases mitochondrial membrane potential (around 20–50 mV more negative inside mitochondria). If uncoupling occurs (increased proton conductance of the mitochondrial inner membrane), the effect of oligomycin is blunted or even abolished. Consistently, the relative differences concerning the measurements of parameters 1, 2, 4, and 5 mentioned

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above between coupled and uncoupled cells are amplified in presence of oligomycin. It is to be mentioned that within cells it could happen that the mitochondria work in "reverse mode" with the mitochondrial ATPsynthase working as an ATPase hydrolysing ATP and pumping protons. Then addition of oligomycin would depolarize mitochondria. The maintenance of such a situation is dependent upon a glycolytic activity able to create an ATP/ADP ratio higher than that reached by mitochondrial activity. Although a prominent glycolytic activity may explain it, it is likely to be caused by a mitochondrial defect: inhibition of respiratory chain (poisoning/hypoxia) or uncoupling. The simultaneous examination of variations in membrane potential, respiration and ATP/ADP ratio may be required to clarify the effect of oligomycin and to evidence uncoupling. The measurement of mitochondrial membrane potential within cells is dependent on the use of probes with which artefacts are always possible. Consequently, the sole observation of a decreased accumulation of a potential sensitive probe within cells is not sufficient to evidence faithfully uncoupling.

Biochemistry with purified UCPs or isolated mitochondria could not solve unambiguously the problem either: most biochemical studies made with the new UCPs produced data that were consistent with these proteins being able to increase the passive proton permeability of the mitochondrial inner membrane [10–14]. Unfortunately, uncoupling (proton transport) was also recorded with other proteins (transporters of the mitochondrial inner membrane distinct from the UCPs) having already well defined activity such as the ADP/ATP translocase [15]. Therefore, whenever observed in these experiments, the debate would turn into the physiological relevance of this uncoupling function observed *in vitro*. Alternatively, one may propose that uncoupling function is shared by a large number of related proteins that perform also/alternatively other more specialized tasks [16,17]. The model established by the group of Cambridge tallies now with this possibility: This model states that mitochondrial superoxide production causes damage to mitochondrial phospholipids and the resulting oxidized lipid species are activators of the UCP protonophoric activity [12]. However, the involvement of the adenine nucleotide translocase is gradually getting more important [15,18,19]. This model has one defect: the preventive action of the uncoupling is taking place after significant damage (lipid peroxidation) had occurred [20]. The possibility of uncoupling through the activation of a proton transport activity of the adenine nucleotide translocase was promoted by the group of V. Skulachev in the eighties [21]. In a sense after years of efforts we have been put "back in USSR". The explanation of the uncoupling effect of fatty acids (or other hydrophobic compounds) on the adenine nucleotide translocase resides in a mechanism known as fatty acid cycling [9,21]. It is important to recall that there are serious objections to the possibility that this fatty acid cycling mechanism would explain the uncoupling activity of UCP1 in brown adipose tissue [8,22,23].

More recently, it was shown that presence of UCP2 and UCP3 significantly increased the intensity of mitochondrial calcium uptake within cells [24]. This uptake is performed by a yet unknown mitochondrial calcium uniporter (MCU). However UCPs could not be identified as the MCU, as introduction of the mammalian UCPs into yeast failed to provide a "mammalian MCU" to the mitochondria of this organism [24]. The acceptance of the concept that UCP2 and UCP3 are intrinsic components of the MCU is now dependent on its confirmation by other groups. Anyway, it is important to realize that if UCP2/3 presence increases the calcium entry into mitochondria it is expected that the calcium would not accumulate indefinitely. The calcium extrusion out of mitochondria is dependent upon membrane potential driven transporters (not ATPases), which ultimately obtain energy from the use of the proton gradient (membrane potential). Therefore, a futile cycle of calcium is expected to take place that would ultimately increase the re-entry of the proton pumped by the respiratory chain and thus increase respiration without increasing ATP turnover. This is uncoupling. The difference with the activity of a protonophoric UCP is an increased complexity: two or more transporters are involved. With the important reservation associated with the effects of modified calcium signaling, the consequences of this futile cycle of calcium in terms of mitochondrial bioenergetics (1 to 5 above) would be identical with that of a protonophore.

Although we have introduced the term UCP2 [25] and provided data substantiating its uncoupling activity through the use of the yeast expression system [10], we failed to find evidence for the uncoupling due to the endogenous UCP2 in mice [26]. This could have been interpreted as a failure to provide the right activating conditions to the proton transport activity of the protein [10–14]. We would like to suggest the possibility that UCP2 or UCP3 have an activity distinct from uncoupling that is physiologically relevant.

3. New UCPs and metabolism

In our recent articles we have examined successively: 1) the consequences of recombinant expression human UCP3 in CHO cells [27]; 2) how expression of avian UCP (avUCP) modifies the activity of yeast mitochondria [14,17]; 3) the differences between mouse embryonic fibroblasts (MEF) derived from wild type mice or *Ucp2*-knock out mice in terms of cellular proliferation [28]; 4) finally, we have compared the oxidative metabolism of glutamine in the macrophages of *Ucp2*-knock out mice and of their wild type controls [29].

The conclusion of these studies could be summarized here: 1) UCP3 modifies bioenergetic parameters in the mitochondria of CHO cells, these modifications did not appear consistent with uncoupling and seemed to be associated with situations where glucose was present in the medium. 2) Avian UCP expressed in yeast appeared similar to UCP2: a genuine uncoupling activity is activated by retinoic acid but the concentrations of retinoic acid that have to be used would make its physiological significance doubtful. However, in the absence of activation, avUCP induced changes in yeast mitochondria bioenergetics that were interpreted to be the result of a combination of avUCP activity with that of other transporters naturally present in yeast mitochondria. 3) MEF from Ucp2-KO mice differ from their wild type counterparts in that they have a higher glucose oxidation rate and a faster dividing activity. 4) In the presence of glutamine the macrophages of Ucp2-KO mice exhibit impaired homeostasis of the glutamine derived metabolites which is associated with a partially impaired mitochondrial bioenergetics.

Fatty acids are known stimulators of the expression of the UCP2/3 mRNA [30,31] and glutamine stimulates the translation of the UCP2 mRNA and therefore increases the mitochondrial content of UCP2 [32]. The fact that glutamine participates to the replenishment of Krebs cycle in C4 compounds has been demonstrated experimentally [33]. It can be concluded that stimulators of UCP2 expression (fatty acids and glutamine) offer the possibility of feeding a complete TCA cycle in the absence of glucose (Fig. 1). It is therefore consistent with the expression data to propose that the role of UCP2 would be to promote oxidation of glutamine and fatty acids rather than that of the pyruvate derived from glucose (Figs. 1 and 2) [17,27,28].

In other words, we propose a "metabolic hypothesis" concerning the physiological role of the new UCPs (UCP2, UCP3 in mammals), this metabolic hypothesis is schematized in Figs. 1 and 2. It does not need an uncoupling (proton transport) activity of the UCP but is likely to be explained by another transport activity yet to be determined. This means that the UCP action could be visible although uncoupling does not take place. Something already recorded by us and others, with a moderate overexpression of UCP3 [34] or with endogenous levels of UCPs [29,35]. The metabolic hypothesis is therefore an alternative to the "uncoupling hypothesis". The two hypothesis are not mutually exclusive. Our bet is that "time is on *our* side" and that the metabolic hypothesis will be increasingly accepted as appropriate to explain the modifications brought about by UCP2/3 activity manipulation while with the uncoupling hypothesis "*we* can get no satisfaction"[36].

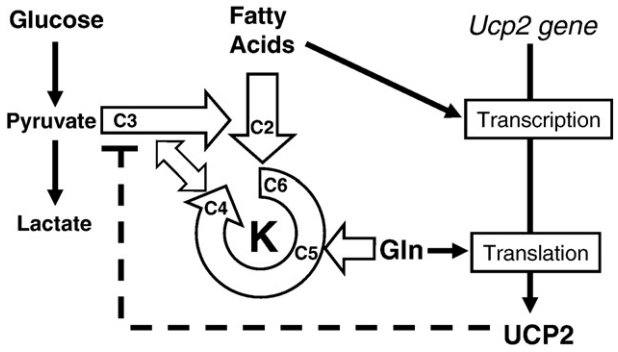

Fig. 1. The oxidative metabolism of glucose, fatty acids and glutamine is schematized around the Krebs cycle (K). The relationships between fatty acids, glutamine and UCP2 expression are shown on the right.

Actually, publications from other groups [37–39] attracted attention to the metabolic consequences of UCP2/3 (over)expression. We would like to emphasize two points by which our proposal diverges from theirs: 1)While it is not always explicitly formulated, the common interpretation of these publications is that UCP2/3 are "fatty acid oxidation genes", which appears somehow contradictory with the expression pattern. Our proposal is that UCP2/3 are "non glucose/pyruvate oxidation genes" and we will examine later why this fits better with the expression pattern. 2) The uncoupling is usually called for either by reference to literature, or because measurements suggest its occurrence. Our hypothesis states that UCP2/3 act primarily without uncoupling mitochondria.

Of course, this metabolic hypothesis does not concern UCP1 whose physiological relevance is the mitochondrial uncoupling necessary for cold induced thermogenesis in the brown adipose tissue. The occurrence of this thermogenesis in adult human is now more widely accepted than before [40].

4. Hypothesis: new UCPs avoid the irreversible degradation of C3 to C2

UCP3 expression is more intense in glycolytic fibers [41] and the pattern of expression of UCP2 (with the highest expression level in spleen) [42] appears also consistent with a predominance of expression in glycolytic cells. These expression patterns appear contradictory with the data indicating that in cultured cells presence of the endogenous UCP2 is associated with a more intense oxidation of fatty acids while its absence is associated with an increased oxidation of glucose [28,34,37–39,43]. To resolve this contradiction it is necessary to emphasize the fact that our hypothesis supposes that UCP2 is neither necessary to the oxidation of fatty acids nor promotes directly this oxidation but UCP2 inhibits pyruvate (glucose) mitochondrial oxidation (Fig. 1). This brake on glucose oxidation allows (or is compensated by) fatty acids usage (Fig. 2).

In this scheme, anaerobic glycolysis could take place from glucose to pyruvate in a glycolytic cell but the mitochondrial degradation of a C3 (pyruvate) product into a C2 (acetyl CoA) is avoided. In other words, cells dependent on glycolysis could still obtain anaerobic energy but when UCP2/3 are expressed these cells would not go beyond the irreversible step from C3 to C2 that makes gluconeogenesis impossible. This obviously makes sense in a complex organism where this glycolytic energy metabolism and the gluconeogenesis from the C3 products occur in different cells. The gain in terms of energy efficiency is limited. It just guarantees that glycolytic cells could still be fed with glucose but their limited mitochondrial oxidation capacities use substrates other than the pyruvate/lactate they produce. This could explain why no dramatic phenotype of the *Ucp2*/*Ucp3* knock out mice have been reported with regard to resistance to starvation.

A consequence of this hypothesis would be that inside cells, the presence of a new UCP could lead to a situation where glycolysis (from glucose to pyruvate) could take place, but most of the pyruvate would not be used by mitochondria (Figs. 1 and 2). The inhibition of glycolysis by oxygen is known as the Pasteur effect, it is a result of the higher efficiency of mitochondrial oxidation to provide ATP compared to glycolysis. When glucose is the main substrate, mitochondrial efficiency is dependent upon the oxidation of pyruvate by mitochondria. Our hypothesis proposes that presence of UCP2/3 results in a decreased affinity of mitochondria for pyruvate. Therefore, for a given mitochondrial pyruvate oxidation, UCP2/3 containing cells would have a higher rate of glycolysis, and therefore a blunted Pasteur effect. In hepatocytes (that do not express any of the mammalian UCPs) it has been checked experimentally, through metabolic control analysis, that the pyruvate oxidation exerts a significant control over the mitochondrial ATP production [44]. Our hypothesis predicts that this control would be decreased in UCP2/3 expressing cells. Opposed to the Pasteur effect is the Warburg effect where cells maintain a high lactate production in presence of oxygen. In a recent publication the Warburg effect in leukemia cells was associated with the presence of UCP2 [45]. In this publication, the link between UCP2 presence and uncoupling appeared loose enough and it leaves open the possibility that UCP2 would not act (solely) by uncoupling respiration.

In other words: expression of UCP2 or UCP3 would allow a cell to remain glycolytic within an aerobic organism. A conclusion consistent with the expression pattern of both proteins.

5. From sparing to sensing: glucose sensing a main target

While UCP2 would have few consequences in quantitative terms (amount of energy) it would deeply influence the way glucose would control internal ATP supply which would impact glucose signaling. This is fully verified by the set of data that pointed to UCP2 as a brake for glucose signaling [2,3]. In these articles UCP2 action has been

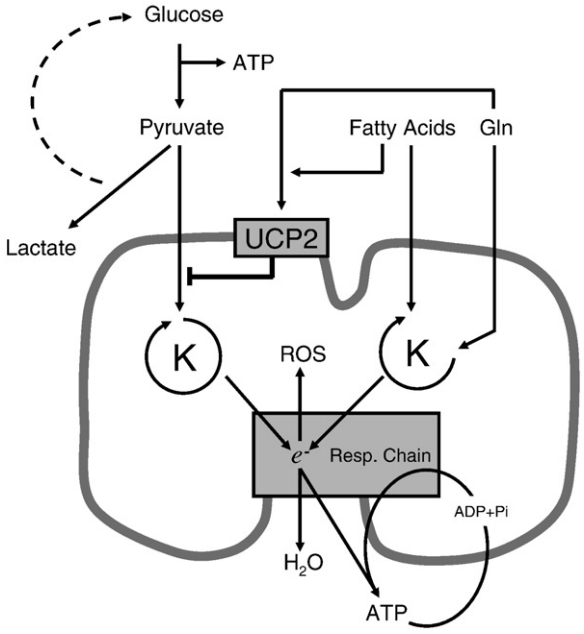

Fig. 2. A graphic representation of our "metabolic hypothesis" see text. The inner membrane of mitochondria is schematized as a thick grey line with UCP2, the Krebs cycle (K) and the respiratory chain (Resp. Chain). The Krebs cycle is schematized twice attempting to highlight the differences between glucose (Pyruvate) or Glutamine + Fatty acids oxidation. The respiratory chain means here all the OXPHOS system: complexes 1–5 plus transporters for Pi and ATP/ADP. Stimulation is schematized by an arrow, inhibition by a T ended line. The biochemical activity of the UCP2 responsible for a decreased pyruvate oxidation is yet to be elucidated.

interpreted in terms of uncoupling: an uncoupler would decrease ATP production whereas glucose would increase it.

Based on the data obtained in [28] one could make the rough calculation shown in Fig. 3: in the presence of UCP2 about 67% of ATP is originating from glucose oxidation whereas in its absence it is 89%. Accordingly, within a cell devoided of UCP2 the dependence of ATP on glucose is higher and any signaling linked to ATP/ADP ratio would be essentially controlled by glucose. In contrast, when UCP2 is present glucose has lost some of its strength on the control of the ATP/ADP ratio. Therefore, within this metabolic hypothesis, UCP2 does not counteract directly glucose signaling by lowering the ATP/ADP ratio whatever the source of metabolic energy is. Instead, UCP2 impacts specifically on the increase in ATP/ADP ratio linked to an intracellular increase in glucose. It therefore lowers the amplitude of glucose signaling. This would modify glucose sensing without compromising the energy level (ATP/ADP ratio ensured by other substrates) of the cell. In the case of pancreatic beta cells, a high UCP2 expression level would remain compatible with an elevated insulin secretion (explained by a high ATP/ADP ratio?). The elevated UCP2 expression level would just make the cell insensitive to variation in glucose level [3]. In this respect, an explanation taking into account the metabolic hypothesis appears more consistent with the phenotype of *ob*/*ob* mice in which an elevated expression of UCP2 is associated with high insulin levels in serum and loss of the sensitivity of the islet to glucose [2]. In absence of irreversible damages to the pancreatic beta cells, lowering UCP2 expression or inhibiting its transport activity will increase glucose sensitivity [46]. The inclusion of this effect into the metabolic theory [46] requires that the inhibition of the other transport involved is similar to that of the proton transport. This is true for the known anionic conductances of UCPs [22,47,48].

A

	Palmitate oxidation	
	Ucp2-KO	Ucp2-WT
Pecqueur et al. fig(4)	500	1500
Relative values	1	3

	Glucose oxidation	
	Ucp2-KO	Ucp2-WT
Pecqueur et al. fig(4)	2000	1500
Relative values	4	3

B

	ATP production		
	Ucp2-KO	Ucp2-WT	2 (KO-WT)
from palmitate	129	387	-258
from glucose	4 x N	3 X N	+258
	N= 258		
from Palmitate	129	387	
from Glucose	1032	774	
Sum	1161	1161	
% from glucose	89%	67%	

C

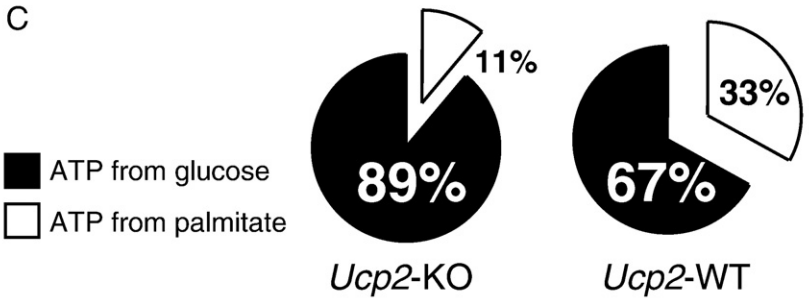

Fig. 3. Estimation of the relative influence of glucose and of fatty acids on ATP production in *Ucp2*-KO or *Ucp2*-WT mouse embryonic fibroblasts (MEF). (A) Reminding of the values found in our previous article [28]. (B) Calculation in terms of ATP, the assumptions are *i*) that the ATP turnover is the same in both types of MEF and *ii*) that the decrease in palmitate oxidation in *Ucp2*-KO MEF is entirely compensated by the observed increase in glucose oxidation. Then the calculation is made for the time needed to oxidize 1 palmitic acid in *Ucp2*-KO MEF which according to basic biochemical principles should give 129 ATP. (C) Graphic representation of the results.

6. Oxygen radicals

The link between UCP2/3 and oxygen radicals is well established [1,5,49]. Concerning the production of oxygen radicals in macrophages [1], the elucidation of the role of the UCP2 has been shown to be relatively indirect [50]. At the same time some reports indicated that the activity of macrophages was strictly correlated to the substrate (fatty acid or glucose) they used [51]. Consequently, it does not need to take into account the mitochondrial production of superoxide to explain the observations made with macrophages. Actually, the participation of the new UCPs to the control of mitochondrial ROS production has been contested [52]. However, the metabolic hypothesis could perfectly explain how the new UCPS could decrease mitochondrial ROS production in absence of uncoupling, hence without impacting on energetic efficiency.

Fig. 2 shows an integrated view of our hypothesis. The share of control of glucose and alternate substrates on ATP production is made visible as well as a simplified view on ROS production. Schematically, electrons given to the respiratory chain could follow two different routes: the normal route leads them to water and is associated with phosphorylation of ADP+Pi into ATP. In this scheme uncoupling is the opening of a second "normal route" for electrons where the energy of oxidation is lost, additive to (but also competitive with) the ATP production. The ROS production is caused by single electrons escaping the normal route. It is easily understandable that the ROS production would depend upon the balance between the number of electrons that could follow the normal route and the number of electrons getting into the respiratory chain. So that for a given ATP turnover the ROS production would depend on the entry of electrons into the respiratory chain. It could be easily concluded that UCP2 by putting a brake on the entry of glucose into the oxidation pathway lowers the ROS production.

Here also the expression of the UCPs in glycolytic cells makes sense. In these cells pyruvate production is intense. This pyruvate would put a enormous redox pressure on the mitochondrial respiratory chain with the risk of a high ROS production. UCP2 by counteracting pyruvate admission into the Krebs cycle would protect the mitochondria inside glycolytic cells. Similarly to what is said above concerning glucose sensing, this defense against oxygen radicals within glycolytic cells would impact directly on the origin of the problem (pyruvate abundance) and it would not jeopardize through uncoupling the other sources of metabolic energy. Obviously, this protection is needed more when alternate mitochondrial substrates are available and the stimulation of UCP2 expression by fatty acids and glutamine remains understandably independent of the conditions where glucose needs to be spared. In fact it appears likely that the lowering of ROS production when a combination of substrates is present would be the primitive role for the UCPs. Its relevance to glucose sparing and signaling gained importance as the complexity of organisms increased.

This role in glycolytic cells appears highly relevant to the consequences of ischemic shocks.

7. UCPs and ischemic shocks

Several reports pointed to the UCPs overexpressed by genetic engineering as remarkably able to protect cells from the consequences of ischemic [5,53,54] or even traumatic events [5]. Here again uncoupling has been called for as an explanation. Uncoupling would limit mitochondrial membrane potential during reperfusion, this would reduce both ROS production and calcium uptake. In this respect the recent proposal that mitochondrial calcium entry is increased when UCP2 or UCP3 are overexpressed appears at odds with their protective effect on reperfusion. In the case of UCP1 we have clearly evidenced that its protective role in the reperfused heart of transgenic mice requires a quantitatively significant uncoupling that maintains the respiratory activity to its maximal value although ATP turnover is reduced [54]. Concerning UCP2 [5] while the authors could record spectacular protective effects, the demonstration of uncoupling could not be provided and authors eventually turned to other explanations including ROS partitioning [5].

During ischemia cells have no other choice than to turn to anaerobic glycolysis to obtain energy. When reperfusion takes place the mitochondria of these cells will be put in a situation comparable to that of glycolytic cells (see above). This is likely to be aggravated by the fact that the ischemic cells, whose survival is most required are differentiated cells where mitochondria are much more abundant than in the "normal glycolytic cell". Therefore, the potency of ROS production is enormous and so are the potential damages associated to it. Consequently, during the ischemic period turning to glycolysis is a condition for survival but it becomes a threat during reperfusion due to the ROS production derived from the increased glucose usage. In this scheme the value of UCP2/3 as a brake for pyruvate entry into mitochondria might be considerable and it may explain while spectacular effects could be recorded in absence of uncoupling of respiration. Moreover, the protective effect of uncoupling is contested [55,56].

8. Conclusion

While the effect of UCP2/3 on cellular metabolism is firmly established we still miss conclusive evidence about the transport activity that explains this effect. In this respect both hypotheses (metabolic or uncoupling) still require experimental support. It may appear as provocative concerning the uncoupling hypothesis. However, in many publications uncoupling remains an interpretation based on observations made in cells/animals correlated with the existing biochemical knowledge obtained *in vitro*. It must also be emphasized that the observation that chemical uncouplers mimick some of the effects of a UCP inside a cell is not a proof that the UCP acts by uncoupling. Mild uncoupling was proposed initially independently (before) the discovery of the new UCPs, reviewed in [57], as a mean to prevent superoxide formation in mitochondria. When these new UCPs arose, the mild uncoupling found conveniently two genes and consequently mild uncoupling was associated to their expression even if it could not be quantitatively detected. At the opposite, when we examined more carefully our models where the effect of the new UCP could have been interpreted as the result of an uncoupling activity, we failed to demonstrate evidence for uncoupling and in fact accumulated evidence against it. Actually, our work concerning glutamine metabolism [29] shows a situation where UCP2 changes mitochondrial metabolism in the absence of any uncoupling activity. Accordingly, it appears now impossible to us to explain the physiological relevance of the new UCPs uniquely by their uncoupling activity as observed *in vitro*.

This has led to the formulation of a metabolic hypothesis fully consistent with knowledge concerning the control of the expression of the UCP2. In the absence of uncoupling, another transport activity of the UCP may be involved. In its simplest form the metabolic hypothesis would be explained by a uniport for anionic pyruvate lowering mitochondrial affinity for pyruvate as membrane potential increases [27]. UCP2 or UCP3 are obvious candidates to be this pyruvate anionic uniport but the proof is far from being produced. Elucidation of the role of the new UCPs in absence of uncoupling would need the study with resolutive metabolic analysis (NMR?) of the internal pathways for metabolites in presence/absence of the new UCP coupled with re-examination of the various solutes that could be transported by UCP2/3.

We have suggested here that this metabolic hypothesis explains why important modifications linked to UCP2 activity have been recorded although in some cases clear evidence for mitochondrial uncoupling was missing. Moreover, the metabolic hypothesis appears

more specific and would not lead to a waste of energy which is a obligatory consequence of uncoupling. This would not mean that uncoupling by the new UCPs is not relevant to physiology but it would rather be a secondary/side effect of these proteins [58], while their role in the control of glucose/pyruvate metabolism would explain their existence in so many different organisms.

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