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Cardiolipin content is involved in liver mitochondrial energy wasting associated with cancer-induced cachexia without the involvement of adenine nucleotide translocase

Cloé Mimsy Julienne [a], Marine Tardieu [a,c], Stéphan Chevalier [a,b], Michelle Pinault [a,b], Philippe Bougnoux [a,b,c], François Labarthe [a,b,c], Charles Couet [a,b,c], Stéphane Servais [a,b,d,*,1], Jean-François Dumas [a,b,*,1]

[a] INSERM UMR1069, "Nutrition, Growth and Cancer", F-37032 Tours, France
[b] Université François-Rabelais, F-37032 Tours, France
[c] CHRU Bretonneau, F-37044 Tours, France
[d] IUT, F-37044 Tours, France

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ABSTRACT

Cancer-induced cachexia describes the progressive skeletal muscle wasting associated with many cancers leading to shortened survival time in cancer patients. We previously reported that cardiolipin content and energy-wasting processes were both increased in liver mitochondria in a rat model of peritoneal carcinosis (PC)-induced cachexia. To increase the understanding of the cellular biology of cancer cachexia, we investigated the involvement of adenine nucleotide translocator (ANT) in mitochondrial energy-wasting processes in liver mitochondria of PC and pair-fed control rats and its interactions with cardiolipin in isolated liver mitochondria from healthy rats exposed to cardiolipin-enriched liposomes. We showed in this study that functional ANT content was decreased in liver mitochondria from PC rats but without any effects on the efficiency of ATP synthesis. Moreover, non-phosphorylating energy wasting was not affected by saturating concentrations of carboxyatractylate (CAT), a potent inhibitor of ANT, in liver mitochondria from PC rats. Decreased efficiency of ATP synthesis was found in normal liver mitochondria exposed to cardiolipin-enriched liposomes, with increased non-phosphorylating energy wasting, thus mimicking mitochondria from PC rats. However, the functional ANT content in these cardiolipin-enriched mitochondria was unchanged, although non-phosphorylating energy wasting was reduced by CAT-induced inhibition of ANT. Finally, non-phosphorylating energy wasting was increased in cardiolipin-enriched mitochondria with substrates for complexes 1 and 2, but not for complex 4. In conclusion, increased energy wasting measured in liver mitochondria from rats with cancer cachexia is dependent on cardiolipin but independent of ANT. Interactions between ANT and cardiolipin are modified when cancer cachexia occurs.

1. Introduction

A large number of cancer patients suffer from cancer-induced cachexia, a complex and multifactorial metabolic syndrome characterized by weight loss (depletion of muscle with or without loss of adipose tissues). Cancer cachexia provokes a severe reduction in autonomy and quality of life and also markedly increases chemotherapy toxicity and therefore decreases patient survival [1,2]. This poor nutritional status is also responsible for 20% of deaths per se (immobility, cardiac and respiratory failure) in cancer patients [3]. Treatment is therefore needed to improve quality of life and reduce mortality. However, the precise molecular biology mechanisms involved in cancer cachexia remain to be determined [4].

In cancer patients, such weight loss is characterized by a negative energy balance due partly to increased whole body energy expenditure [4]. The liver, a highly metabolic and energy-demanding organ [5], might be involved in the increased energy expenditure associated with cancer cachexia. First, liver mass and resting energy expenditure are increased in cachectic patients with advanced colorectal cancer [6]. Secondly, the energy metabolism of liver mitochondria is impaired in several rodent models of cancer cachexia [7–9]. Thirdly, we previously demonstrated a significant decrease in the efficiency of ATP synthesis taking place in the liver mitochondria in a rat model of peritoneal

Abbreviations: ANT, adenine nucleotide translocase; BSA–FAF, bovine serum albumin–fatty acid free; CAT, carboxyatractylate; CL, cardiolipin; M-CAT, mitochondria treated with carboxyatractylate; M-CL, mitochondria treated with cardiolipin; M-PE, mitochondria treated with phosphatidylethanolamine; NAO, 10-N-nonyl acridine orange; PC, peritoneal carcinosis; PE, phosphatidylethanolamine

* Corresponding authors at: INSERM UMR1069, Laboratoire de Nutrition, Croissance et Cancer, Faculté de Médecine, Bâtiment Dutrochet, 10 Boulevard Tonnellé, F-37032 Tours, Cedex 1, France. Tel.: +33 247 36 60 59; fax: +33 247 36 62 26.
E-mail addresses: stephane.servais@univ-tours.fr (S. Servais), jean-francois.dumas@univ-tours.fr (J.-F. Dumas).
[1] These authors contributed equally to this work.

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carcinosis (PC) with advanced cachexia [10]. This suggests that liver mitochondria of cachectic rats need to consume more nutrients than liver mitochondria of healthy rats to maintain a satisfactory level of ATP synthesis.

The ATP required by respiring cells is mainly produced by mitochondrial oxidative phosphorylation [5]. In this process, phosphorylation of ADP to ATP by F_1F_0ATPase is coupled to oxygen consumption, providing the final reductor of nutrients in the mitochondrial respiratory chain. However, the coupling (i.e. the efficiency of ATP synthesis) is not perfect, and some of the energy potential of nutrients is dissipated as heat instead of being converted into ATP. Such energy wasting is inversely correlated with the efficiency of ATP synthesis but positively correlated with the quantity of nutrients used to satisfy the demands of ATP.

We found that the reduced efficiency of ATP synthesis measured in liver mitochondria of rats with cancer cachexia was correlated with an increase in cardiolipin (a specific phospholipid of the mitochondrial inner membrane) content (+55%, $R^2 = 0.64$, $p < 0.05$) [10]. The liver-specific energy wasting associated with increased energy expenditure during cancer cachexia may thus partly be explained by changes in quantity of cardiolipin and molecular species [10].

Cardiolipin plays a critical role in the normal structure and function of the adenine nucleotide translocator (ANT) [11] and it has been hypothesized that ANT might represent a downstream target of pathological states involving changes in cardiolipin metabolism [12]. ANT is an important component of the mitochondrial machinery of ATP synthesis because of its intrinsic activity of transporting ADP/ATP across the inner membrane. Such transport is energy costly due to the associated partial collapse of the electrochemical gradient established by the electron transport chain [13]. In addition, ANT is involved in both basal and fatty acid-induced non-phosphorylating energy-wasting processes [14,15]. ANT may also be involved in energy wasting as reported in a model of weight loss induced by glucocorticoid treatment [16]. Whether ANT could play such a role in the specific situation of weight loss associated with cancer cachexia required exploration.

The study reported here was therefore carried out to investigate the involvement of ANT in energy wasting observed in isolated liver mitochondria from rats suffering from cancer cachexia induced by PC. Complementary experiments were performed to study the potential functional interaction between ANT and cardiolipin in isolated liver mitochondria exposed to cardiolipin-enriched liposomes.

2. Materials and methods

2.1. Experimental design

The study was performed in accordance with the French guidelines for the care and use of animals and was approved by the local Ethics Committee ("Comité d'Ethique en Expérimentation Animale Centre Val de Loire") (Authorization #2010-34). *Cancer cachexia experiment*: rats (healthy immunocompetent Berlin-Druckrey IX, Charles River, L'Arbresle, France) were divided into 2 groups as previously described [10]: i) a peritoneal carcinosis group (PC; $n = 6$), fed ad libitum, which received a single intraperitoneal (IP) injection of 10^6 of PROb cells in 1 ml of culture medium and ii) a control group ($n = 6$) in which rats weight-matched to PC rats received an IP injection of 1 ml of culture medium without PROb cells. Control rats were pair-fed (each rat from the control group was paired with a rat from the PC group) in order to discriminate between the effects of anorexia and PC on the parameters measured in this study. Body weight, food intake, behaviour and physical appearance were recorded every other day. PC rats were sacrificed at an advanced state of cachexia (severe anorexia apparent, significant loss of skeletal muscle mass and of perirenal white adipose tissue mass in comparison to control rats) as previously described [17]. Livers were rapidly removed after sacrifice and mitochondria were isolated as previously described [10]. *Cardiolipin enrichment experiment*: one batch of isolated liver mitochondria (from healthy Sprague–Dawley, Charles River, L'Arbresle, France) was separated into 4 groups: i) untreated mitochondria (M-control) and mitochondria treated with ii) carboxyatractylate (CAT), a specific inhibitor of ANT (M-CAT), iii) cardiolipin (M-CL) and iv) phosphatidylethanolamine (M-PE).

2.2. Preparation of liver mitochondria

Livers were rapidly removed after sacrifice and mitochondria were isolated by differential centrifugation in an ice-cold isolation medium (250 mM sucrose, 1 mM EGTA and 20 mM Tris/HCl, pH 7.4) [10]. Protein concentration was determined using the Bicinchoninic Acid Assay kit (Interchim, France).

2.3. Mitochondrial oxygen consumption

Oxygen was measured using a Clark oxygen electrode (Oxygraph, Hansatech, France). Mitochondria (0.5 mg of protein/ml) were incubated in a respiratory reaction medium at 37 °C, consisting of 120 mM KCl, 5 mM KH_2PO_4, 1 mM EGTA, 2 mM $MgCl_2$, and 3 mM Hepes (pH 7.4) supplemented with 0.3% (w/v) BSA–FAF, and saturated with room air. The substrate and inhibitor concentrations were 5 mM succinate and 2.5 μM rotenone, respectively. The ATP synthesis-related oxygen consumption was initiated by the addition of 300 μM ADP. The non-phosphorylating oxygen consumption was obtained by the addition of oligomycin (2 μg/mg of protein).

2.4. Measurement of functional ANT content

Functional ANT content was determined by titrating ATP synthesis-related oxygen consumption with increasing non-saturating concentrations of CAT [18]. The mitochondrial content of ANT was determined by extrapolating the linear part of the titration curve to obtain the amount of CAT required to inhibit ATP synthesis-related oxygen consumption completely. The experimental conditions were: mitochondria (1 mg protein/ml), succinate (5 mM), rotenone (2.5 μM), ADP (1.5 mM), 0.3% (w/v) BSA–FAF and 37 °C.

2.5. Efficiency of ATP synthesis by mitochondria

The kinetic response of oxygen consumption to changes in ATP synthesis was determined at 37 °C in a respiratory reaction medium (120 mM KCl, 5 mM KH_2PO_4, 1 mM EGTA, 2 mM $MgCl_2$, 3 mM Hepes, pH 7.4) supplemented with 0.3% (w/v) bovine serum albumin, and saturated with room air [10]. The experimental conditions were: mitochondria (1 mg protein/ml), succinate (5 mM), rotenone (2.5 μM), glucose (20 mM) and ATP (125 μM). The rate of ATP synthesis was modulated and followed by glucose 6-phosphate accumulation using an ADP regenerating system based on hexokinase (0–0.5 U) plus glucose and ATP. Oxygen was concomitantly measured by oxygraphy (Hansatech, France). To test the involvement of ANT, CAT was added at a non-saturating concentration (100 pmol/mg of protein) in some experiments. This dose is able to inhibit ATP synthesis-related oxygen consumption to about 16 ± 1%.

2.6. Non-phosphorylating energy wasting by mitochondria

The kinetic response of oxygen consumption in response to a variation in membrane potential in non-phosphorylating conditions (without ATP synthesis) was determined as previously described [19]. The experimental conditions were: mitochondria (0.5 mg protein/ml), succinate (5 mM), rotenone (2.5 μM), oligomycin (2 μg/mg mitochondrial protein), 0.3% (w/v) BSA–FAF, nigericin (80 ng/ml) and 37 °C.

A saturating concentration of CAT (2 μM) was used to test the involvement of ANT in non-phosphorylating energy wasting. To test the effects of fatty acids on non-phosphorylating oxygen consumption,