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Cinnamon intake alleviates the combined effects of dietary-induced insulin resistance and acute stress on brain mitochondria☆,☆☆,★

Karine Couturier[a,b], Isabelle Hininger[a,b], Laurent Poulet[a,b], Richard A. Anderson[c,1], Anne-Marie Roussel[a,b], Frédéric Canini[d,e], Cécile Batandier[a,b,*]

[a]Laboratoire de Bioénergétique Fondamentale et Appliquée, Université Grenoble Alpes, F-38041 Grenoble cedex France
[b]U1055 - INSERM, F-38041 Grenoble France
[c]Beltsville Human Nutrition Research Center, United States Department of Agriculture, Beltsville, MD USA
[d]Département Neurosciences & Contraintes Opérationnelles, Institut de Recherche Biomédicale des Armées (IRBA), BP73, 91223 Brétigny-sur-Orge cedex, France
[e]Ecole du Val de Grâce, 1 place Alphonse Laveran, 75230 Paris, France

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Abstract

Insulin resistance (IR), which is a leading cause of the metabolic syndrome, results in early brain function alterations which may alter brain mitochondrial functioning. Previously, we demonstrated that rats fed a control diet and submitted to an acute restraint stress exhibited a delayed mitochondrial permeability transition pore (mPTP) opening. In this study, we evaluated the combined effects of dietary and emotional stressors as found in western way of life. We studied, in rats submitted or not to an acute stress, the effects of diet-induced IR on brain mitochondria, using a high fat/high fructose diet (HF^2), as an IR inducer, with addition or not of cinnamon as an insulin sensitizer. We measured Ca^{2+} retention capacity, respiration, ROS production, enzymatic activities and cell signaling activation. Under stress, HF^2 diet dramatically decreased the amount of Ca^{2+} required to open the mPTP (13%) suggesting an adverse effect on mitochondrial survival. Cinnamon added to the diet corrected this negative effect and resulted in a partial recovery (30%). The effects related to cinnamon addition to the diet could be due to its antioxidant properties or to the observed modulation of PI3K-AKT-GSK3β and MAPK-P38 pathways or to a combination of both. These data suggest a protective effect of cinnamon on brain mitochondria against the negative impact of an HF^2 diet. Cinnamon could be beneficial to counteract deleterious dietary effects in stressed conditions.

Keywords: Polyphenols; Brain; Mitochondria; Acute stress; Insulin resistance; Permeability transition pore

1. Introduction

Insulin resistance (IR), an epidemic that is associated with the increased incidence of obesity, metabolic syndrome and Type 2 diabetes, strikes both developed and emerging countries and constitutes an important socio-economic public health concern. Diet-induced IR and combined oxidative stress have been associated also with early brain dysfunctions and behavioural alterations. The brain insulin pathway is involved in multiple regulatory mechanisms including neuronal survival and cognitive processes like learning and memory [1]. In addition, neurodegenerative diseases such as Alzheimer's disease or mental pathologies, such as depression, have also been associated with brain IR [2].

Since mitochondria are involved in redox regulation and cellular metabolic activities, oxidative alterations result in mitochondrial dysfunctions. In addition, emotional stress could be an aggravating factor of the metabolic syndrome and, consequently, of brain alterations. In contrast, some nutritional factors such as polyphenols could counteract the IR-induced brain alterations and, therefore, be neuroprotective through both their antioxidant and insulin-potentiating properties.

In daily life, stressful conditions are often associated with deleterious dietary habits. Independently, chronic stress exposure induces, by itself,

Abbreviations: mPTP, mitochondrial Permeability Transition Pore; CRC, Calcium Retention Capacity; C, Control; C/S, Control with Stress; C+Cn, Control plus Cinnamon; C+Cn/S, Control plus Cinnamon with Stress; HF^2, High Fat/High Fructose; HF^2/S, High Fat/High Fructose with Stress; HF^2+Cn, High Fat/High Fructose plus Cinnamon; HF^2+Cn/S, High Fat/High Fructose plus Cinnamon with Stress; ETC, Electron Transport Chain; IR, Insulin-Resistant or Insulin-Resistance; P/T, Phosphorylated/Total protein ratio; AMPK, Adenosine Monophosphate-Activated Protein Kinase; PI3-K, Phosphatidylinositol 3-Kinase; GSK-3ß, Glycogen Synthase Kinase 3 beta; CsA, Cyclosporine A; Rot, Rotenone; DNP, Dinitrophenol; DCIP, Dichloroindophenol

☆ In memoriam to Professor Xavier Leverve (1950–2010)

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* Corresponding author at: Laboratoire de Bioénergétique Fondamentale et Appliquée, Université Grenoble Alpes, INSERM U1055; BP 53X, F-38041 Grenoble cedex, France. Tel.: +33-476635637; fax: +33-4765142-18.
E-mail address: Cecile.Batandier@ujf-grenoble.fr (C. Batandier).

[1] Currently PolyChrom Technology, Edgewater, MD.

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peripheral insulin resistance [3]. However, the molecular mechanisms underlying the relationship between stress and brain IR remain not totally understood [4].

Dietary habits impact the brain mitochondrial functioning. Although negative impacts for health of high fructose intakes are well established, fructose consumption is steadily increasing in western countries. High fructose intake results in oxidative stress and decreased insulin sensitivity and is a leading cause of IR that causes the metabolic syndrome and Type 2 diabetes [5]. Furthermore, the metabolic syndrome and Type 2 diabetes are associated with an increased risk of brain dysfunction [6,7]. In parallel, excess consumption of fat, as often found in western diets, has also well-known consequences on brain oxidative stress, with increased production of reactive oxygen species (ROS) [8] and dysfunctional mitochondria [7].

Conversely, dietary conditions might exert neuroprotective effects [9]. Among protective nutrients, high intakes of fruit and vegetables, rich in polyphenols have been reported as beneficial for the brain [10,11]. A protective effect on brain mitochondria has been suggested related to their antioxidant properties [12]. Recent studies have elucidated the neuroprotective effects of insulin in brain linked to redox biology [13], suggesting that a part of these effects might be related to an improvement in insulin sensitivity.

Since our group has previously reported that, in overweight subjects with increased oxidative stress and decreased insulin sensitivity, cinnamon (Cn) polyphenols acted positively in decreasing oxidative stress and improving insulin sensitivity [14], we studied the impact of Cn on diet-induced IR under stressed conditions.

Cn polyphenols are dietary biologically active components that exhibit, antioxidant and insulin potentiating properties [15,16]. At the cell level, Cn polyphenols modulate mitochondrial processes [biogenesis, apoptosis, electron transport chain (ETC), ATP synthesis] [17]. At the systemic level, they regulate blood glucose and lipid levels, preventing Type 2 diabetes [18–20]. Furthermore, they have been reported to exert neuroprotective effects in ischemic injury [21], with the mitochondria as targets [22].

Dietary and psychological stresses are accompanied by rapid changes in mitochondrial functioning. We reported previously that animals fed a control diet and submitted to an acute restraint stress, exhibited a delay in mitochondrial permeability transition pore (mPTP) opening of brain mitochondria [23]. This change was associated with a partial inhibition of Complex I respiratory chain and a modulation in AMPK signaling pathway. These data suggest that restraint stress could act in stimulating cell-survival processes and stimulate mitochondrial adaptive mechanisms.

Brain mitochondria might be one of the key actors between stress and dietary intake, IR and the occurrence of brain dysfunctions. Brain mitochondria are not only fountains of energy for the cell; they are also critically involved in neuronal survival. They are essential regulators of cellular Ca^{2+} and producers of ROS, the spearheads of oxidative signaling. Indeed, the mPTP, a key component of the membrane permeability transition, is involved in Ca^{2+} matrix regulation through its transient opening and in cell death pathways through its final opening. Oxidative stress participates to its opening [24], and conversely, its opening is associated with an increase in mitochondrial ROS production [25].

The present work is part of a larger study [15,23,26,27] aiming to determine if (a) IR-induced brain alterations are related to oxidative stress and mitochondrial dysfunction and (b) IR-induced brain alterations could be modulated by bioactive antioxidant and insulin sensitizer components of the diet like polyphenols from Cn.

Here, we investigated the combined effects of dietary-induced IR and acute stress on brain mitochondria with a focus on the potential beneficial effects of Cn consumption.

The interactions between dietary and emotional stresses were assessed using an animal model of IR, the HF^2 rat, [26] with or without the addition of Cn and submitted or not to an acute restraint stress [27]. As previously published by our group, this model exhibited a significant decrease in brain insulin sensitivity assessed by mRNA expression alterations in insulin signaling pathway [15].

We studied brain functioning through a bioenergetics approach with mitochondria as the focal point. We measured (a) functional parameters of brain mitochondria [Ca^{2+} retention capacity (CRC), respiration, ROS production, enzymatic activities] and (b) signaling parameters to assess relationships between mitochondria and cells in a double stress situation, in presence or not of Cn.

2. Materials and methods

2.1. Animals and diets

Eighty-four weaning male Wistar rats (Charles River, L'Arbresle, France), weighing 50–75 g were housed in individual cages in a temperature controlled room (22 °C) with a 12-h light/12 h dark cycle. All experimental procedures were reviewed and approved by the Institutional Ethic Committee for Animal Care (CRSSA, Protocol N 2008/02.1 accepted in December 2008). The rats were maintained and handled in accordance with the Guide for the Care and Use of Laboratory Rats (NIH 1985).

The diets were purchased from SAFE® (Augis, France). Two types of diets were used: Purina chow as the control diet with (C+Cn) or without (C) 20 g/kg of Cn powder and a high-fat/high-fructose diet (HF^2) containing 45% fructose and 20% lard to induce IR [26] with (HF^2+Cn) or without (HF^2) 20 g/kg of Cn powder. The Cn powder (Cinnamomum burmannii) was purchased from McCormick Spice Co., Baltimore, MD, USA.

2.2. Experimental design and stress procedure (Fig. 1)

Rats were adapted and fed the control diet for 2 weeks. They were then randomly divided into four groups and fed the control diet (C) *ad libitum* for 12 weeks, the control diet plus Cn (C+Cn), the HF^2 and the high fat/high diet plus Cn (HF^2+Cn). At the end of 12 weeks, the rats were exposed or not to a restraint stress, a well-established emotional stressor in rodents before being sacrificed. The rats were therefore distributed into eight experimental groups and analyzed as followed: control diet with (C/S, n=8) and without (C, n=10) stress, control diet plus Cn with (C+Cn/S, n=11) and without (C+Cn, n=10) stress, HF^2 diet with (HF^2/S, n=10) and without (HF^2, n=13) stress and HF^2+Cn diet with (HF^2+Cn/S, n=10) and without (HF^2+Cn, n=12) stress.

For the restraint stress, stressed rats underwent forced immobilization for 30 min in a plastic wire mesh restrainer adjusted to individual size to ensure a psychological strain without any body compression. They could not move, causing intense discomfort without apparent pain. The control rats stayed undisturbed in their home cage until sacrifice.

The rats exposed or not to stress were weighed then sacrificed by decapitation. Brains were quickly removed. The cerebellum was immediately frozen in liquid nitrogen and stored at −80 °C for western blot analyses, and the forebrain was immediately used for mitochondrial analyses. Unfortunately, decapitation was associated with hemolysis that did not allow us to assess glucocorticoid levels. The strong stress effect on the blood glucocorticoid levels was measured on another series of animals conditioned in the same conditions (diet, restraint stress and operator) [27]. All experimental animal procedures have been described previously in detail [23].

2.3. Brain mitochondrial functions

2.3.1. Preparation of brain mitochondria

Forebrain mitochondria were prepared according to Rosenthal method. Mitochondrial protein concentration was determined by a bicinchoninic acid assay (BCA proteins assay, Pierce®, Rockford, IL, USA) using bovine serum albumin as a standard.

2.3.2. mPTP opening

Changes in mPTP opening were assessed by the CRC test that measures the capacity of mitochondria to store repeated amount of calcium until the opening of mPTP. The free Ca^{2+} was measured fluorimetrically in the presence of 0.25-μM Calcium Green-5 N® (Life Technologies SAS, Saint Aubin, France) with excitation and emission wavelengths set at 506 and 530 nm, respectively, with a PTI Quantamaster® C61 spectrofluorimeter (Photon Technology International, Edison, NJ, USA) equipped with magnetic stirring and thermostatic controls (30 °C). The ETC substrate was 5-mM succinate (S). As appropriate, 1-μM CSA (C) or 1-μM rotenone (R) was also added to the medium. After 1-min incubation, measurement was started and after 1-min signal stabilization, 5-μM Ca^{2+} pulses were successively added at 1-min intervals until the opening of mPTP, as indicated by the release of Ca^{2+} into the medium. In order to quantify the increase in CRC induced by stress, three experimental conditions as no modulator or CsA, a well-documented modulator of mPTP, either rotenone, a specific Complex I inhibitor were combined to calculate the average value of change induced by stress (Fig. 2E).

2.3.3. Mitochondrial respiration

Mitochondrial oxygen consumption was measured polarographically at 30 °C using a Clark-type oxygen electrode in a Mitocell S200®(Strathkelvin Instruments Limited, North Lanarkshire, Scotland). Oxygen consumption rate of mitochondria (1 mg) was