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Bioenergetics of Neurological Disease and Aging

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*Stipend Awardees
The mitochondrial sirtuin Sirt3 protects neurons from oxidative injury

Sun Hee Kim, Huafei Lu, Conrad Alano

Dept. of Neurology, San Francisco VA Medical Center/UCSF, San Francisco, CA

Oxidative stress occurs with excessive generation of reactive oxygen species (ROS), and is a principal factor in cellular damage that occurs in ischemic stroke and other neurological injuries. We have previously reported that the mitochondrial sirtuin isoform Sirt3 reduced ROS production, prevented mitochondrial depolarization, and reduced neuronal death induced by exposure to NMDA. New preliminary data implicates Sirt3 in protecting from an in vitro ischemia model using oxygen and glucose deprivation (OGD). We hypothesized that Sirt3 reduces oxidative injury by enhancing endogenous antioxidant defense systems. Overexpression of Sirt3 in mitochondria protected against OGD, whereas Sirt3 silencing or knockout exacerbated OGD injury. Sirt3 protection is mediated by deacetylation of isocitrate dehydrogenase 2 (Idh2) and superoxide dismutase-2 (SOD2). We further demonstrated that deacetylation of Idh2 and SOD2 by SIRT3 is required to reduce ROS production and oxidative damage. These data indicate that increasing SIRT3 activity enhanced antioxidant defenses, and promoted neuronal survival from simulated ischemic injury in cultured mouse cortical neurons.
Ethylmalonic acid impairs mitochondrial succinate and malate uptake in rat brain mitochondria

Alexandre U. Amaral¹, Cristiane Cecatto¹, Estela N. B. Busanello¹, César A. J. Ribeiro¹, Daniela R. Melo², Guilhian Leipnitz¹, Roger F. Castilho² and Moacir Wajner¹,³

¹ Dept of Biochemistry, ICBS, UFRGS, Porto Alegre, RS, Brazil; ² Dept of Clinical Pathology, FCM, UNICAMP, Campinas, SP, Brazil; ³ Medical Genetics Service, HCPA, Porto Alegre, RS, Brazil

Short chain acyl-CoA dehydrogenase (SCAD) deficiency and ethylmalonic encephalopathy (EE) are metabolic disorders biochemically characterized by tissue accumulation of ethylmalonic acid (EMA). Although the patients affected by these disorders present severe neurological symptoms, the pathophysiological mechanisms of brain damage are poorly unknown. In the present work we investigated the in vitro effects of EMA on important parameters of mitochondrial bioenergetics in rat brain mitochondria supported by succinate, malate, or glutamate plus malate. The parameters evaluated were states 3 and 4 respiration, respiratory control ratio (RCR), uncoupled state, succinate dehydrogenase (SDH) activity, mitochondrial membrane potential (ΔΨm) and mitochondrial dicarboxylate transporter activity. We verified that EMA inhibited state 3 respiration, RCR and uncoupled state in succinate- and malate-, but not glutamate plus malate-supported mitochondria. Furthermore, EMA mildly increased state 4 succinate-respiring mitochondria. We also observed that methylmalonic acid (MMA), malonic acid (MA) and butylmalonic acid (BtMA) had a similar effect on state 3 respiration. In addition, EMA-, MMA- and BtMA-, but not MA-induced inhibitory effect on succinate oxidation, was significantly minimized by nonselective permeabilization of mitochondrial membranes provoked by alamethicin. In this context, MA was the only tested compound that reduced SDH
activity in an apparently competitive manner. It was also demonstrated that EMA markedly inhibited succinate and malate transport through the mitochondrial dicarboxylate transporter. Finally, the $\Delta \Psi_m$ was reduced by EMA and MA, but not by MMA, using succinate as substrate, whereas none of these compounds was able to alter this parameter using glutamate plus malate as substrate. Our results strongly indicate that EMA impairs succinate and malate uptake through the mitochondrial dicarboxylate carrier and that it may be relevant to the pathophysiology of SCAD and EE.

Keywords: short chain acyl-CoA dehydrogenase deficiency, ethylmalonic encephalopathy; ethylmalonic acid

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Ab-heme model for mitochondrial dysfunction and energy hypometabolism in brain of Alzheimer’s disease

Hani Atamna, Jeanette Mackey, Kristen Canteralla

Department of Basic Sciences, Neuroscience, The Commonwealth Medical College, Scranton, PA

The metabolism of Amyloid-β (Aβ) peptides become abnormal in the brain of Alzheimer’s disease (AD) patients. Although the view that Aβ causes neurodegeneration in AD is most popular, the molecular mechanism is lacking. We directed our research at investigating the molecular mechanism of Aβ’s neurotoxicity. We identified a heme-binding motif in human Aβ (huAβ) and provided experimental evidence that huAβ tightly binds with heme, forming huAβ-heme complex. We showed that the binding of heme with huAβ depletes intracellular regulatory heme, thus interfering with the metabolism of energy, redox, and iron. huAβ binding with heme explains the declines in mitochondrial complex IV, mitochondrial function, synaptic function, and increases oxidative stress (e.g., increase H2O2). Interestingly, we also found that Aβ-heme complex exhibits a peroxidase activity and uses H2O2 to oxidize various metabolites. We have identified the amino acids (1-16 aa) as the heme-binding motif in huAβ. Species that develop AD-like neuropathology share an identical amino acid sequence with huAβ including the heme-binding motif. Rodents, on the other hand, lack AD-like neuropathology. The amino acid sequence of rodent Aβ (roAβ) is identical to huAβ, except for three amino acids within the heme-binding motif. Interestingly, roAβ does not bind heme or form peroxidase. Furthermore, the lipophilic amino acids 17-42 aa of Aβ are identical among all the species regardless of the presence or absence of AD-like neuropathology, consistent with the fact that both huAβ and roAβ equally form aggregates. These phylogenic differences in heme-binding motif may explain, in part, why human, but
not rodents, develop AD with age. huAβ-heme could serve as a diagnostic marker and drug target to delay the onset of AD. We will present additional recent research investigations from my lab and discuss future directions in the light of huAβ-heme model.
Compartmentation of energy metabolism in neurons and astrocytes

Lasse K. Bak et al.

Dept. of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Neurons and astrocytes contain the primary energy-generating pathways of glycolysis and mitochondrial oxidative metabolism. Yet, the regulation of these pathways seems to differ not only between the two cell types but even at the level of the single cell; that is, neurons and astrocytes are divided into different functional and metabolic compartments with little cross-talk between these. The poster will present some recent and older data pointing to the existence of cellular compartmentation and discuss some possible explanations for this phenomenon at the cellular and molecular levels.
Mitochondrial CB1 receptors regulate neuronal energy metabolism

Giovanni Bénard 1–3,11, Federico Massa 1,3,11, Nagore Puente 4, Joana Lourenço 1,3,5,10, Luigi Belloccchio 1,3, Edgar Soria-Gómez 1,3, Isabel Matias 1,3, Anna Delamarre 1,3, Mathilde Metn-Laurent 1,3, Astrid Cannich 1,3, Etienne Hebert-Chatelain 1,3, Christophe Mulle 3,5, Silvia Ortega-Gutiérrez 6, Mar Martín-Fontecha 6, Matthias Klugmann 7,8, Stephan Guggenhuber 7, Beat Lutz 7, Jürg Gertsch 9, Francis Chaouloff 1,3, María Luz López-Rodríguez 6, Pedro Grandes 4, Rodrigue Rossignol 2,3 and Giovanni Marsicano 1,3

1 INSERM U862, NeuroCentre Magendie, EndoCannabinoids and NeuroAdaptation, Bordeaux, France 2 Laboratoire Maladies Rares: Génétique et Métabolisme, Bordeaux, France 3 University of Bordeaux, Bordeaux, France 4 Department of Neurosciences, Faculty of Medicine and Dentistry, Basque Country University, Leioa, Spain 5 Laboratoire Physiologie Cellulaire de la Synapsee, Centre National de la Recherche Scientifique UMR 5091, Bordeaux, France 6 Department of Organic Chemistry, Complutense University, Madrid, Spain 7 Institute of Physiological Chemistry, University Medical Center of the Johannes Gutenberg University, Mainz, Germany 8 Translational Neuroscience Facility, Department of Physiology, School of Medical Sciences, University of New South Wales, Sydney, NSW, Australia 9 These authors share authorship 10 present address: European Brain Research Institute, "Rita Levi-Montalcini" Foundation, Rome, Italy

The mammalian brain is one of the organs with the highest energy demands, and mitochondria are key determinants of its functions. Here we show that the type-1 cannabinoid receptor (CB1) is present at the membranes of mouse neuronal mitochondria (mtCB1), where it directly controls cellular respiration and energy production. Through activation of mtCB1 receptors, exogenous cannabinoids and in situ endocannabinoids decreased, complex I enzymatic activity and respiration in neuronal mitochondria. In addition, intracellular CB1 receptors and mitochondrial mechanisms
contributed to endocannabinoid-dependent depolarization-induced suppression of inhibition in the hippocampus. Thus, mtCB1 receptors directly modulate neuronal energy metabolism, revealing a new mechanism of action of G protein–coupled receptor signaling in the brain.
The production of reactive oxygen species is a key event in excitotoxic neuronal death, and we have previously demonstrated a primary source of superoxide is the enzyme NADPH oxidase. In cortical neuronal cultures incubation with NMDA (100µM for 30min) resulted in significant production of superoxide (as measured by dHet fluorescence). This increase in superoxide production was dependent on the activation of an atypical PKC (Brennan et al, 2009). However, the mechanism by which NMDA receptor activation results in activation of this aPKC is poorly characterized. We demonstrate here that calcium influx through NR2B-containing NMDA receptors is required for NADPH oxidase superoxide production. Application of the calcium ionophore, ionomycin, induced comparable increases in calcium and resulted in mitochondrial membrane depolarization but not in increases in superoxide production. Further, we found that inhibition of phosphoinositide 3-kinase (PI3K) is sufficient to inhibit phosphorylation of a key NADPH oxidase subunit p47^phox, superoxide production and cell death. These results suggest that calcium influx through NR2B results in a PI3K-dependent phosphorylation of the atypical PKCzeta, phosphorylation and translocation p47^phox, and superoxide production from the enzyme NADPH oxidase.
Reversible mitochondrial aging as an early oxidized redox state that precedes macromolecular ROS damage in non-transgenic and AD-model mouse neurons

Gregory J. Brewer¹,², and Debolina Ghosh²

¹ Dept. of Neurology and ² Dept. of Medical Microbiology, Immunology and Cell Biology, Southern Illinois University School of Medicine, Springfield, Illinois USA

The brain depends on redox electrons from NADH to produce ATP and oxyradicals (ROS). Since ROS damage and mitochondrial dysregulation are prominent in aging and Alzheimer’s disease (AD) and their relationship to redox state is unclear, we wanted to know whether an oxidative redox shift precedes these markers and leads to macromolecular damage in the aging mouse brain neurons and in a mouse model of AD. We used the 3xTg-AD mouse model that displays cognitive deficits beginning at 4 months. Hippocampal/cortical neurons were isolated across the age-span and cultured in common nutrients to control for possible hormonal and vascular differences. We found an increase of NAD(P)H levels and a reductive shift in redox state in non-transgenic neurons until middle age, followed by a decline (oxidized shift) in old age. The 3xTg-AD neurons maintained much lower resting NAD(P)H and a more oxidized redox state after 4 months, but the NADH regenerating capacity continuously declined with age beginning at 2 months. These redox characteristics were partially reversible with nicotinamide, a biosynthetic precursor of NAD+. Nicotinamide also protected against glutamate excitotoxicity. Compared to non-transgenic neurons, 3xTg-AD neurons possessed more mitochondria/neuron and lower glutathione levels which preceded age-related increases in ROS levels. These glutathione deficits were again reversible with nicotinamide in 3xTg-AD neurons. Surprisingly, low macromolecular ROS damage was only elevated after 4
months in the 3xTg-AD neurons if anti-oxidants were removed. The present data suggest that a more oxidized redox state and a lower antioxidant glutathione defense can be dissociated from neuronal ROS damage, changes that precede the onset of cognitive deficits in the 3xTg-AD model.
Disruption of bioenergetics by phytanic acid in cerebellum of young rats

Busanello EN¹, Amaral AU¹, Zanatta A¹, Tonin AM¹, Viegas CM¹, Wajner M¹,²

¹ Departmento de Bioquímica, Universidade Federal do Rio Grande do Sul 2 Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre

Refsum disease is a peroxisomal disorder biochemically characterized by elevated phytanic acid (Phyt) tissue concentrations. Cerebellar ataxia, polyneuropathy and progressive retinitis pigmentosa occur in most affected patients. Although Phyt has cytotoxic effects on brain cells, little is known about its action in cerebellum and the pathophysiology of this disorder is practically unknown. In the present study we investigated Phyt effects on important parameters of cellular bioenergetics in rat cerebellum in order to clarify the pathogenesis of the cerebellar ataxia in this disorder. Mitochondrial enriched fractions, homogenates and synaptic membranes preparations were obtained from cerebellum of 30-day-old rats. Various parameters of energy metabolism were determined in the presence of 10-200 µM Phyt. First, we measured the state 3 respiration in mitochondria and observed that Phyt reduced this parameter, reflecting a role as a metabolic inhibitor. In attempt to clarify Phyt-specific action, we evaluated the activities of the isolated complexes of respiratory chain in homogenates. We observed that complexes I-III, II and II-III activities were strongly inhibited by Phyt, without affecting complex IV activity. These data suggest that Phyt impairs the substrate oxidation in the respiratory chain. We then examined state 4 respiration, RCR(respiratory control ratio), NAD(P)H levels and membrane potential in enriched mitochondria. State 4 respiration was increased, whereas RCR, NAD(P)H levels and membrane potential were reduced by Phyt using both glutamate plus malate or succinate as substrates, suggesting that this
metabolite acts as an uncoupler of oxidative phosphorylation. Next we evaluated the influence of Phyt on Na+-K+-ATPase activity, which consumes high amounts of the ATP generated in the central nervous system, in synaptic membranes preparations. Phyt strongly inhibited this activity, indicating that neurotransmission, which depends on normal Na+-K+-ATPase activity, is possibly impaired by Phyt. Taken together, these data provide experimental evidence that Phyt provokes impairment of cerebellum energy metabolism, acting as an uncoupler of oxidative phosphorylation and as a metabolic inhibitor, besides affecting neurotransmission. It is therefore postulated that bioenergetics deficit may possibly contribute to the pathophysiology of the cerebellar symptoms present in Refsum disease.

Keywords: Refsum disease, phytanic acid, energy metabolism, rat cerebellum
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Objective metabolic phenotyping of knock-in mice reveals novel dysfunctional pathways in Huntington’s disease

Jeffrey B. Carroll ¹,³, Elisa Fossale ¹, Rory Weston ³, Amy Deik ², Jolene Guide ¹, Vanessa C. Wheeler ¹, Ihn Sik Seong ¹, Jong-Min Lee ¹, Clary B. Clish ² and Marcy E. MacDonald ¹,²

1 Center for Human Genetic Research, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA 02114, USA.
2 Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA.
3 Behavioral Neuroscience Program, Department of Psychology, Western Washington University.

Symptoms of Huntington’s Disease are linked to the dysfunction of specific neural circuits, particularly in the frontal cortex and basal ganglia. A bewildering array of mechanisms has been proposed to underlie this critical dysfunction. We hypothesize that a better understanding of the normal roles of the huntingtin protein (HTT) and how these functions are modulated by poly-glutamine expansion will inform better therapeutic strategies. To prioritize phenotypes observed in mouse models of HD, we consider that onset of symptoms of HD is 1) dominant and 2) CAG tract length-dependent. With these criteria in mind, we note that HD is associated with dominant, CAG-dependent, cellular and organismal metabolic dysfunction.

We are evaluating an allelic series of CAG knock-in mice to understand HTT’s role in cellular and organismal metabolism, beginning with an analysis of wild type (Q7/Q7) and heterozygous mutant (Q111/Q7) mice. To study gene-environment interactions, we are feeding mice both normal and high-fat diets. These mouse studies are complemented by a study of genetically identical cultured CAG knock-in neuronal progenitor cells. First, static metabolite profiles of >150 identified metabolites are being established using liquid chromatography tandem mass spectrometry (LC-MS/MS) in
the striatum, cerebellum, liver, white fat, brown fat and plasma from wild type (Q7/Q7) and heterozygous mutant (Q111/Q7) mice. Next, metabolic flux from [U-13C]-D-glucose to CO2, lactate, glutamate, and lipids is being determined in matched tissues.

These studies reveal tissue specific alterations in a number of metabolic pathways of interest, particularly in the striatum, the region of the brain most vulnerable to pathology in HD. Observed changes include reduced levels of organic osmolytes in the striatum of mutant mice. Altered balance across the membrane of taurine, a prototypical osmolyte, is also observed in vitro. These taurine alterations in vitro are associated with reduced viability after hypotonic challenge. Given the recently demonstrated role for wild type HTT in regulating cellular response to hypo-osmotic stress in Dictyostelium discoideum, we suggest that poly-glutamine expansion in HTT may alter osmotic homeostatic mechanisms in the striatum, which may contribute to HD pathogenesis.
A reduction in the activity of alpha-ketoglutarate dehydrogenase complex decreases matrix substrate-level phosphorylation and prompts respiration-impaired mitochondria towards extramitochondrial ATP consumption

Gergely Kiss, Csaba Konrad, Anatoly A. Starkov, Hibiki Kawamata, Giovanni Manfredi, Steven F. Zhang, Gary E. Gibson, M Flint Beal, Vera Adam-Vizi and Christos Chinopoulos

1 Department of Medical Biochemistry, Semmelweis University, Budapest, 1094, Hungary
2 Weill Medical College Cornell University, New York, NY, 10021, USA
3 Weill Cornell Medical College/Burke Medical Research Institute, White Plains, NY, 10605, USA

Provision of succinyl-CoA by the alpha-ketoglutarate dehydrogenase complex (KGDHC) is essential for generation of matrix ATP (or GTP) by substrate-level phosphorylation catalyzed by succinyl-CoA ligase. A decline in KGDHC activity has been associated with a number of neurodegenerative diseases. The focus of this association has been biased towards the diminished provision of reducing equivalents, and the extent of reactive oxygen species formation. Here we show that transgenic mice with a deficiency in the dihydrolipoyl succinyltransferase (DLST) or dihydrolipoyl dehydrogenase (DLD) subunit of KGDHC exhibiting a 25-40% decrease in activity, prompted respiration-impaired i) isolated, ii) in situ synaptic and iii) in situ neuronal somal mitochondria towards extramitochondrial ATP consumption. This was attributed to a shift in the reversal potential of adenine nucleotide translocase towards more negative values due to diminished matrix substrate-level phosphorylation, thus causing the translocase to reverse prematurely in
respiration-impaired mitochondria of DLST+/- and DLD+/- mice. These results were further corroborated by the finding that mitochondria from wild-type mice respiring on substrates that supported substrate-level phosphorylation, exhibited higher ADP-ATP exchange rates compared to those obtained from DLST+/- and DLD+/- mice. We propose that KGDHC-associated pathologies are subserved by the inability of respiration-impaired mitochondria to rely on “in-house” mitochondrial ATP reserves.
α-Syntrophin knockout mice given unilateral acoustic activation retain more label derived from [6-14C] glucose in the activated inferior colliculus than control mice.

Nancy F. Cruz¹, Kelly K. Ball¹, Stanley C. Froehner ², Marvin E. Adams², and Gerald A. Dienel¹

1 Dept. of Neurology, University of Arkansas for Medical Sciences. Little Rock, AR, USA 2 Dept. Physiology and Biophysics, University of Washington, Seattle, WA, USA

α-Syntrophin is component of the dystrophin scaffold-protein complex that serves as an adaptor for recruitment of membrane channels, receptors, kinases, and other proteins to the cytoplasmic side of plasma membranes. In astrocytic endfeet, α-syntrophin is associated with aquaporin 4 (AQP4), and knockout (KO) of α-syntrophin causes loss of the polarized distribution of AQP4 at endfeet facing the vasculature, redistribution of AQP4 to other sites in the astrocytic membranes, and interference with water flow between blood and brain and with K+ homeostasis (Neely et al., 2001; Amiry-Moghaddam et al., 2004. Eid et al., 2005). We hypothesize that water movements involving astrocytic endfeet are necessary for and coincident with metabolite release and perivascular fluid flow during brain activation. If true, impairment of water fluxes should impede lactate clearance from brain during activation, and α-syntrophin KO mice would have reduced lactate release during brain activation and greater retention of labeled metabolites of [6-14C]glucose compared to control mice. To test this hypothesis, glucose utilization was assayed with [6-14C] glucose during a unilateral auditory stimulus paradigm (Cruz et al., 2007). On the day of the experiment, adult male mice were anesthetized, arterial and venous catheters inserted, and one eardrum punctured. Three hours later, mice were given a single tone (8 kHz, 103 dB) acoustic stimulus and 10
min later [6-14C]glucose was injected intravenously. Timed samples of arterial plasma were drawn and assayed for 14C and glucose levels, and five min later, the mice were euthanized. Brains were removed, frozen, cut into 20 mm-thick sections, and exposed to x-ray film along with 14C-standards. 14C concentrations were determined in the activated and contralateral inferior colliculus of KO and control mice. The activated/contralateral ratio for 14C level (mean ± SD) in the entire inferior colliculus was significantly (p <0.05) higher for the KO mice (1.17 ± 0.11, n = 5) compared to control mice (1.05 ± 0.02, n = 6). In contrast, the right/left brain ratios for 10 non-auditory structures were similar in both groups and were close to 1.0. These findings support the hypothesis that lactate and other labeled metabolites are retained in activated brain tissue to a greater extent when AQP4 localization at astrocytic endfeet is disrupted.
Brain metabolic dysfunction in response to systemic inflammation and sepsis

Joana C. d’Avila¹, Renata Carnevale¹, Rachel N. Gomes¹, Patricia A. Reis¹, Luiz F. Garcia-Souza², Hugo C. Castro Faria Neto¹, Marcus F. Oliveira² and Fernando A. Bozza³

1 Laboratório de Imunofarmacologia, Instituto Oswaldo Cruz – FIOCRUZ, Rio de Janeiro, RJ, Brasil; 2 Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, RJ, Brasil; 3 Intensive Care Unit, Instituto de Pesquisa Clínica Evandro Chagas, FIOCRUZ, Rio de Janeiro, RJ, Brasil.

Sepsis is frequently associated with systemic inflammatory response syndrome that leads to multiple organ dysfunctions. Acute and long-term brain dysfunctions have been demonstrated both in experimental models and septic patients. Oxygen supply and utilization are critical for organ function and the brain tissue is extremely dependent on glucose and oxygen. We had previously observed that mitochondrial function is impaired in the brain during experimental sepsis, which directly affect brain oxygen utilization. The pathologic mechanisms that lead to septic encephalopathy are not completely understood. In this study we aimed to characterize the effect of systemic inflammation in the brain energy metabolism. We induced systemic inflammation in mice by either endotoxemia or polymicrobial peritonitis as a model of experimental sepsis. As previously observed, mice with systemic inflammation presented hypoglycemia, hyperlactatemia and cognitive impairment. We observed a rapid increase in glucose uptake in the brain of mice with endotoxemia in vivo using Positron Emission Tomography (PET). Remarkably, the increase in glucose uptake occurred earlier in the brain than in other organs, as early as 2h after disease onset. A similar increase in glucose uptake was observed in vitro in brain slices from mice with polymicrobial peritonitis. The brains of mice with experimental sepsis also showed early mitochondrial
dysfunction, as measured by an increase in oxygen consumption, and oxidative stress. The results of the present study show a new metabolic phenotype that occurs in the brain in response to systemic inflammation, characterized by a rapid increase in glucose uptake and mitochondrial dysfunction. This metabolic dysfunction can be related to the development of septic encephalopathy.
Effect of high fat thermolyzed diet on monocarboxylate transporters and energetic metabolism in the hippocampus of diabetic rats and the benefits of the Ω3 polyunsaturated fatty acids

Adriano Martimbianco de Assis¹, Anderson Rech¹, Aline Longoni¹, Ben Hur Mussulini¹, Matheus Augusto Pasquali¹, Cendrine Repond², Diogo Onofre Souza¹, Luc Pellerin², José Cláudio Moreira¹ and Marcos Luiz Santos Perry¹

¹ Department of Biochemistry, Federal University of Rio Grande do Sul, 90035-003, Porto Alegre, Brazil; ² Department of Physiology, University of Lausanne, 1005, Lausanne, Switzerland.

Diabetes mellitus (DM), a state of chronic hyperglycemia, is a common disease and one of the leading causes of morbidity and mortality in developed countries. The aim of this study was to evaluate the effect of high fat thermolyzed diet on monocarboxylate transporters and energetic metabolism in the hippocampus of diabetic rats and the benefits of the Ω3 polyunsaturated fatty acids. Diabetes was induced by an intraperitoneal (i.p.) injection of alloxan (150 mg kg⁻¹ b.w.). Rats were divided into six groups: (1) the control group (C, n=10), which received standard laboratory rat chow; (2) the control diabetic group (D, n=10), which received an injection of alloxan and the same diet as group 1; (3) the high fat thermolyzed diet group (HFTD, n=10), which received a diet enriched with fats that had been heated for 60 min at 180 °C to which a vitamin mixture was added after heating; (4) the high fat thermolyzed diet diabetic group (D+HFTD, n=10), which received an injection of alloxan and the same diet as group 3; (5) the high fat thermolyzed diet + Ω3 polyunsaturated fatty acid group (HFTD+Ω3), which received a diet enriched with fats that had been heated for 60 min at 180 °C to which a vitamin mixture and an omega 3 fatty acid
supplement was added after heating; and (6) the high thermolyzed fat diet + Ω3-polyunsaturated fatty acid diabetic group (D+HFTD+Ω3, n=10), which received an injection of alloxan and the same diet as group 5. After one month of diet the rats were sacrificed by decapitation and the hippocampus quickly removed and processed for further analysis. In the glucose oxidation to CO2 we didn’t observe statistical differences. However, we note that the HFTD diet and diabetes increases the β-hydroxybutyrate oxidation to CO2, and we have a decrease in the lactate oxidation only in D+HFTD group. In western blot, we observed an increase in the MCT1 and MCT2 immunocontent only in the D+HFTD group. A high fat thermolyzed diet decreased lactate oxidation and increased β-hydroxybutyrate oxidation, MCT1 and 2 immunocontent in diabetic rats (D+HFTD) in the hippocampus. We note a relationship between oxidation this substrates and his transporter. The omega 3 was effective in normalizing the monocarboxylic acids metabolism.
Metabolic adaptations in neurons with complex IV deficiency

Francisca Diaz¹, Sofia Garcia¹ and Carlos T Moraes¹,²

¹ Department of Neurology, ² Department of Cell Biology and Anatomy, University of Miami, Miller School of Medicine, Miami, Florida, 33136, USA

We created a neuron specific conditional knockout (KO) mouse model of complex IV (cytochrome c oxidase) deficiency where the ablation of the Cox10 gene in neurons was driven by CamKII-Cre. COX10 encodes a heme a farnesyl transferase indispensable for complex IV assembly and function. The COX10 KO had a reduced life span and the majority of the mice died between 8 and 14 months of age. The COX10 KO displayed behavioral abnormalities with an onset at about 4 months of age, long before their death.

The onset of mitochondrial defect was not accompanied by neuronal death in the COX10 KO mice. A progressive COX deficiency was observed in both cortex and hippocampus showing about 70% of control values at 2 months of age and rapidly declining to 24% by 4 months. The first signs of neuronal degeneration were observed only at about 4 months of age that later developed into a severe cortical atrophy by 8 -10 months.

We investigated early metabolic adaptations responsible for neuronal survival to maintain the energetic crisis produced by the mitochondrial defect. We observed an increased glucose uptake in the COX10 KO brain. Likewise, glycolytic intermediates (G6P, F6P, F1-6BP, DHAP and lactate) and the enzymatic activity of hexokinase were significantly elevated at 3 months of age. Interestingly, glycolysis was modulated by enhanced activity and not by overexpression of glycolytic enzymes.
The COX10 conditional brain KO constitutes an excellent model to study the molecular bases of metabolic adaptations leading to neuronal survival to compensate for an energetic crisis caused by a mitochondrial defect.
Energy metabolism supports both inhibitory and excitatory neurotransmission processes. Using dynamic high resolution 13C magnetic resonance spectroscopy (MRS) in vivo, we now investigated intermediary metabolic pathways contributing to γ-aminobutyric acid (GABA) synthesis and GABAergic neurotransmission in the rat brain. Male Sprague-Dawley rats (267±9 g, n=8) under α-chloralose anesthesia were infused with [1,6-13C]glucose, and 13C MRS was performed at 14.1 T as previously described (Duarte et al., Front Neuroenerg 3:3). Enhanced sensitivity and spectral resolution achieved at high magnetic field, allowed quantifying, for the first time, incorporation of labeling into the three aliphatic resonances of GABA in vivo. Metabolic fluxes were determined with a newly designed mathematical model of brain metabolism that included glial, glutamatergic and GABAergic compartments. GABA synthesis rate was 0.10±0.01 μmol/g/min. Apparent GABAergic neurotransmission, i.e. GABA-glutamine cycle, accounted for 20.3% (0.048±0.004 μmol/g/min) of the total neurotransmitter cycling between neurons and glia. Total brain glucose oxidation was 0.47±0.03 μmol/g/min, from which 39.5% and 8.0% occur in the tricarboxylic acid (TCA) cycle fluxes supporting glutamate-glutamine and GABA-glutamine cycles, respectively. The remainder fraction of glucose oxidation was found to take place in glia, including a contribution of 19.8% from pyruvate carboxylation (0.085±0.007 μmol/g/min). Like in previous studies, exchange fluxes from 2-oxoglutarate to glutamate were in the same order of the respective TCA cycles in the
three compartments. In conclusion, the present results show a substantial metabolic activity in GABAergic neurons and, furthermore, emphasize the active role of glial oxidative metabolism in supporting neurotransmission in both glutamatergic and GABAergic neurons.
Astrocytes become reactive in response to most pathological situations in brain, such as acute brain injuries or chronic neurodegenerative diseases. They contribute to the neuroinflammatory response that accompanies neuronal dysfunction. Reactive astrocytes display morphological changes (hypertrophy, process remodelling) as well as functional changes including alterations in brain energy metabolism (Escartin et al., 2007). Such changes could be detected by diffusion-weighted proton magnetic resonance spectroscopy (DW-MRS) and MRS respectively. This would allow noninvasive monitoring of astrocyte reactivity as an index of neuronal dysfunction. In this study, we used a rat model of astrocyte activation by stereotaxic injection of lentiviral vectors encoding for the cytokine ciliary neurotrophic factor (lenti-CNTF) into the right striatum. The controlateral striatum was injected by a control lentiviral vector that encodes for beta-galactosidase (lenti-LacZ). DW-MRS and MRS data were acquired on a 7T magnet from two voxels placed over each striatum. A diffusion weighted LASER (Localization by Adiabatic Selective Refocusing) sequence with echo time TE=40 ms was used. Concentrations were measured using LCModel software for N-acetyl-aspartate (NAA), myo-inositol (Ins), choline (Cho), glutamate and taurine relative to total creatine (tCr) on the spectrum at b=0, while the apparent diffusion coefficient (ADC) was calculated for all these metabolites (including tCr) from signal measured at b=0 and b= 2000 s/mm². Lenti-CNTF injection promoted a sustained, extensive and selective activation of astrocytes, as
evidenced by overexpression of GFAP and vimentin and cellular hypertrophy. Microglia displayed only limited increase in markers of reactivity and most importantly, neurons displayed unaltered morphological, molecular and electrophysiological features, as described previously (Escartin et al., 2006; 2007; Beurrier et al., 2010). ADC were measured reproducibly for metabolites and for water but were unchanged by astrocyte activation, suggesting undetectable intracellular remodeling. However, MRS evidenced stable changes in metabolite concentration between the two injected striata. In the lenti-CNTF injected striatum, levels of the astrocyte metabolites Ins and Cho were increased whereas levels of the neuronal metabolite NAA were decreased. These results suggest that reactive astrocyte alone, in absence of neuronal degeneration, results in alterations of metabolite concentrations detectable by NMR. These neurochemical changes are potential biomarkers to detect astrocyte activation even without ongoing neurodegeneration.

References
Calcium up-regulates GFAP in activated astrocytes through JNK/c-Jun/AP-1 signaling pathway

Kai Gao, Rui Chao Chai, Feng Jiang, Greg Vatcher and Albert Cheung Hoi Yu*

Neuroscience Research Institute, Peking University; Key Laboratory of Neuroscience (Peking University), Ministry of Education; Key Laboratory of Neuroscience (Peking University), Ministry of Health; Department of Neurobiology, School of Basic Medical Sciences, Peking University, Beijing, China
*Corresponding author
Email: achy@bjmu.edu.cn

Astrocytes are activated under various diseases (including ischemia and physical injuries) resulting in glial scar formation (astrogliosis) in the central nervous system. The reactivation of astrocytes involves energy metabolic and morphology changes with complex underlining mechanisms not well understood. Among these changes, astrogliosis is always accompanied by up-regulation of glial fibrillary acidic protein (GFAP). Using an in vitro scratch injury model and primary cultures of cerebral cortical astrocytes, we observed that a calcium wave was generated in astrocytes immediately after scratching and propagated in a direction away from the wound. Using the Ca2+ blocker BAPTA-AM and JNK inhibitor SP600125, we confirmed that the calcium wave was involved with the phosphorylation of c-Jun N-terminal kinase (JNK), which then phosphorylated the transcription factor c-Jun to facilitate the binding of AP-1 to the GFAP gene promoter region. This resulted in the up-regulation of GFAP transcription. Utilizing an in vivo stab wound model, we confirmed that blocking the calcium signal would suppress the glial scar formation and inhibit the closure of the stab wound. Taken together, our findings indicate that the Ca2+/JNK/c-Jun/AP-1 pathway regulates GFAP expression during astrocyte reactivation. [Supported by National Basic Research Program of China (973 program, 2011CB504400),}
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Scalable fluorescence microscopic assays for mitochondrial physiology

Akos A. Gerencser, David G. Nicholls, Martin D. Brand

Buck Institute for Research on Aging, Novato, California, CA;

Fluorescence microscopic tools are widely used for assessing mitochondrial physiology, however quantitative assay of such parameters are not trivial. Modern, motorized microscope systems established the need for scalable assays that are robust and are able to evaluate biological parameters in an automated, unsupervised manner. We introduce here a cell physiology-oriented software platform implementing novel image processing methods providing analytical tools for mitochondrial physiology. This enables a user friendly access to two of our previously published technologies for low light level, but robust determination of mitochondrial swelling and organelle motion in cultured cells. Mitochondrial swelling is measured by the ratio of fluorescence intensities in high over low frequency spatial band pass filtered copies of the same image. This ratio is highly sensitive to mitochondrial swelling, but insensitive to fission or fusion, motion and overlaps of mitochondria. Organelle transport is assayed by optical flow, featuring instantaneous velocity determination from a pair of images by detecting motion of edges. Optical flow provides velocity vectors; therefore anterograde, retrograde transport and local, wiggling motion can be distinguished. Using advanced image segmentation techniques mitochondria:cytosol volume fractions are measured using MitoTracker Red and calcein-AM with conventional laser scanning confocal microscopy and stereologic considerations. In situ mitochondrial superoxide levels are routinely measured with MitoSOX, but this assay is biased by geometric factors, like cell size and mitochondrial volume density, and is also sensitive to mitochondrial and plasma membrane potentials. Using MitoSOX:MitoTracker Deep Red and advanced image segmentation techniques we have improved
the unbiased detection of mitochondrial superoxide levels. All of these assays are scalable, applicable for imaging in microplates and are accessible through a user friendly interface.
Mitochondrial CB1 receptor impacts neuronal bioenergetics through protein kinase A mediated phosphorylation of the complex I subunit NDUFS2.

Etienne Hebert-Chatelain¹, Giovanni Benard¹,², Tiffany Desprez¹, Luigi Belloccchio¹, Edgar Soria-Gómez¹, Anna Delamarre¹, Mathilde Metna-Laurent¹, Rodrigue Rossignol² and Giovanni Marsicano¹

¹, INSERM U862, NeuroCentre Magendie, EndoCannabinoids and NeuroAdaptation, University of Bordeaux, Bordeaux, France ; ², Laboratoire Maladies Rares: Génétique et Métabolisme, University of Bordeaux, Bordeaux, France

The endocannabinoid system is a critical mediator of neuronal processes as demonstrated by the abundance of the type 1 cannabinoid receptor (CB1) in the brain. Although the brain represents 2 % of the body mass in mammals, it consumes up to 20% of the body’s total energy production. Mitochondria are the most important producers of energy in the brain and are therefore key elements in the regulation of neuronal functions. The CB1 receptor is also present at the level of mitochondrial membranes in mouse neurons where it regulates cellular respiration and energy production. Here we describe the molecular pathway of CB1 regulation of mitochondrial metabolism. Activation of mitochondrial CB1 (mtCB1) decreases mitochondrial respiration in neuronal mitochondria through a G protein dependent mechanism. In fact, mtCB1 activation decreases the activity of soluble adenylyl cyclase (sAC), the levels of cAMP and the activity of the protein kinase A (PKA) in mitochondria. This mtCB1-sAC-cAMP-PKA pathway specifically decreases the enzymatic activity of the complex I of the electron transport system. This decrease of complex I activity is mediated by lower PKA dependent phosphorylation of its subunit NDUFS2. This study shows a novel regulation pathway of neuronal bioenergetics.
through G protein coupled receptor signaling in brain mitochondria.
Brain hypometabolic state in aging: investigating the mechanistic basis and evaluating the potential role of lipoic acid

Tianyi Jiang, Fei Yin, Enrique Cadenas

Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA 90089, USA

Due to the heavy reliance of brain on energy to perform its normal functions such as cognition and memory, impairment of energy metabolism is widely recognized as hallmark of brain aging. However, mechanistic basis leading to brain hypometabolic state in aging is much less known. In this study, Fischer 344 rats are used as the animal model to investigate brain hypometabolic state in terms of a) substrate supply, b) substrate metabolism, and c) regulatory components including cell signaling and transcriptional pathways. As the primary energy source for the brain, glucose uptake declines as a function of age, which could be accounted for partially by the decrease in the expression of neuronal glucose transporters GLUT3. Mitochondria reside in the center of substrate metabolism and its dynamics remodeling is closely related to energy production. Mitochondrial fusion and fission machinery were determined through the assessment of Opa1 and Drp1 expression levels. For the regulatory components, several signaling pathways are examined: PI3K/Akt route of insulin signaling is implicated in neuronal survival and synaptic plasticity; JNK is stress-responsive and translocates to mitochondria, inhibits pyruvate dehydrogenase (PDH) and induces a bioenergetics crisis; AMPK pathway is a well-known cellular energy sensor and metabolic master switch. In addition, PGC1α mediated transcriptional pathway that controls mitochondrial biogenesis was shown to be down-regulated in aging which is associated with reduced mitochondrial energy transducing capacity. The beneficial effect of \( R-(+)-\)lipoic acid on the
energy metabolism of periphery organs has been reported; here we evaluated its potential to modulate the brain metabolic state via thiol-disulfide exchange reactions. Our current data have demonstrated the significant effect of lipoic acid on GLUT expression, PI3K/Akt, JNK and AMPK signaling pathways as well as PGC1a-mediated transcriptional pathway, suggesting its great promise of rescuing brain hypometabolic state in aging.
Functional importance of mitochondrial diaphorases in maintaining phosphorylation potential during inhibition of electron transport chain.

Gergely Kiss, Csaba Konrad, Vera Adam-Vizi and Christos Chinopoulos

Department of Medical Biochemistry, Semmelweis University, Budapest, 1094, Hungary

During tissue anoxia, the electron transport chain becomes inoperable. Under these conditions, mitochondrial matrix substrate-level phosphorylation becomes the only means of intramitochondrial ATP provision. Substrate level phosphorylation is maintained by the succinyl-CoA ligase reaction. Formation of ATP and succinate requires provision of succinyl-CoA, mostly arising from the ketoglutarate dehydrogenase complex (KGDHC) reaction. However, the question arises, which is the source of NAD+ maintaining KGDHC operation during inhibition of the respiratory chain? Here we report that mitochondrial matrix diaphorases utilize endogenous quinones for the regeneration of NAD+ from NADH. Diaphorases are enzymes that reduce a large variety of endogenous substrates, most notably quinones, generating NAD+ from NADH in the process. We also provide evidence that these reduced quinones are re-oxidized by members of the electron transport chain most likely complex III, releasing protons in the intermembrane space, without the necessity for oxidizing NADH or other reducing equivalents. Our findings may lend enormous support to the concept that dietary provision of substances with the ability to accumulate in mitochondria and being substrates for the diaphorases may be tissue-protective in energy crisis, as it occurs in pathological conditions.
Comparison of exogenous alternative fuels on respiration by rat cortical neurons

Melissa D. Laird, Pascaline Clerc, Brian Polster and Gary Fiskum

Department of Anesthesiology and the Shock, Trauma, and Anesthesiology Research (STAR) Organized Research Center, University of Maryland School of Medicine, Baltimore, Maryland

Prolonged and excessive elevation of intracellular Ca2+ and subsequent mitochondrial bioenergetic dysfunction are mediators of glutamate excitotoxicity. One approach toward neuroprotection for excitotoxicity is administration of fuels for aerobic energy metabolism that could potentially overcome inhibition of glucose-based energy metabolism. This study tested the hypothesis that exogenous pyruvate, lactate, acetyl-L-carnitine (ALCAR), and β-hydroxybutyrate (BHB), can stimulate neuronal O2 consumption in vitro either in the absence or presence of excitotoxic glutamate receptor stimulation. Experiments were performed with rat cortical neurons at day in vitro (DIV) 7 and 14 to determine if developmental age influences neuronal respiratory responses to excitotoxicity or to the presence of alternative biofuels. The presence of these biofuels (1.25 – 10.0 mM) in artificial CSF containing 5 mM glucose had no effect on basal O2 consumption rate (OCR) measured with the Seahorse Bioscience cell respirometer; however, the addition of pyruvate, lactate or BHB produced a significant, dose-dependent stimulation of the maximal respiratory capacity measured in the presence of the respiratory uncoupler, FCCP, with both DIV 7 and 14 neurons. The response to these biofuels was also measured after a 30 min exposure to 100 μM glutamate, followed by addition of the receptor antagonists MK801 plus CNQX, and then FCCP. Pyruvate, lactate and BHB each increased the respiratory capacity of DIV 7 neurons exposed to glutamate to the same level observed without glutamate, as expected since maximal OCR by DIV7 neurons was not inhibited by glutamate. In contrast,
glutamate reduced the maximal OCR for DIV14 neurons with glucose alone by 50%. Glutamate exposure also reduced the maximal OCR with glucose plus pyruvate, lactate or BHB; however, these alternative fuels still stimulated respiration to a level higher than that obtained with glucose alone even in the absence of glutamate exposure. These findings indicate that; 1. Basal and maximal OCR for DIV7 and 14 cortical neurons are similar. 2. Pyruvate, lactate and BHB significantly increase maximal OCR by both DIV 7 and 14 neurons to similar extents. 3. Exposure to glutamate for 30 min inhibits maximal OCR for DIV14 but not DIV7 cortical neurons. 4. Pyruvate, lactate and BHB each stimulate the respiratory capacity of DIV14 neurons exposed to glutamate to a lower level than without glutamate but still higher than that sustained in the absence of glutamate when metabolizing glucose alone. These results support the concept that neuroprotection observed with exogenous pyruvate, lactate, or BHB could be mediated by their stimulation of aerobic neuronal energy metabolism. Supported by NIH P01HD16596-25.
Studying effects of glucose transporter isoform 2 on brain metabolic activities using localized 1H MRS and perfusion MRI

Hongxia Lei¹, Frédéric Preitner², Bernard Thorens² and Rolf Gruetter¹,²,³

¹ Dept. of Radiology, University of Geneva, Geneva, Switzerland; ² University of Lausanne, Lausanne, Switzerland; ³ Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

The functionality of glucose transporter isoform 2 (GLUT2) in brain remained not well understood. The aim of this study was to assess neurochemical profiles using localized 1H-MRS and cerebral blood flow using continuous arterial spin labeling (CASL, one of perfusion MRI techniques) on brain of GLUT2/-/ mice and their counter types at 9.4T. Both MR modalities allow us non-invasively studying the effects of GLUT2/-/ on brain, such as hippocampus and hypothalamus. GLUT2/-/ mice were derived along with transgenic re-expression of GLUT1 in the pancreatic β cells as previously described (RIPGLUT1GLUT2/-/). With the approval by the local ethic committee, 19 (16-20th week) mice were studied under 1-2% isoflurane for their breathing rates >100bpm, which had been shown capable of maintaining mice under physiological conditions, such as PCO₂ in the range of 35-45mmHg. Tail bleeds were sampled and measured for glucose levels (Breeze glucose meter) immediately before and right after MR measurements, which were approximately one and a half hours. At 9.4T, localized 1H MRS spectroscopy was applied in hippocampus and hypothalamus using SPECIAL along with sufficient scans (320-480, 20-30mins). CBF was measured using a well-established CASL technique in combination with a home-built actively-detuned system. CBF maps were calculated from 16 paired labeled and controlled images with a labeling efficiency 0.8. MR spectra data were processed and analyzed by referencing to unsuppressed
water signal. Right before the MR measurements, the blood glucose levels in RIPGLUT1GLUT2/- mice were 7.1±2.7mM, slightly lower than that in their WT counterparts, i.e. 10.5±1.5mM. Immediately after the MR measurements, the blood glucose levels of RIPGLUT1GLUT2/- mice reduced to 5.4±1.7mM, which became significantly different when compared to those in the WT mice, 10.0±5.2mM (p=0.0006, student paired t-test). While at euglycemia, CBF was globally higher in RIPGLUT1GLUT2/- mice and significant in hippocampus (p<0.0002). The consistently increased blood flow has been observed here was similar to rat brain under insulin induced hypoglycemia. This suggested a possible role for GLUT2 in glucose sensing mechanism in brain. Other than regional CBFs, taurine, myo-inositol and total creatine, were increased in hippocampus of RIPGLUT1GLUT2/- mice when using localized 1H MRS. The observations at euglycemia suggested that deleting GLUT2 indeed affected brain tissue, i.e. hippocampus, substantially. In summary, GLUT2 played an important role in glucose sensing mechanism in brain and caused elevated blood flow even when the alternation of metabolites in hippocampus indicated a different adaption mechanism other than osmoregulation observed in diabetic animals.
Activity-dependent increases in local oxygen consumption correlate with post-synaptic currents in the mouse cerebellum in vivo.

Claus Mathiesen¹, Kirsten Caesar¹, Kirsten Thomsen¹, Tycho M. Hoogland², Brent M. Witgen¹, Alexey Brazhe³ and Martin Lauritzen¹,⁴.

¹ Department of Neuroscience and Pharmacology & Center for Healthy Aging, University of Copenhagen, Copenhagen N, Denmark; Netherlands Institute for Neuroscience, Amsterdam, the Netherlands
² Biological faculty Moscow State University, Russia; Department of Clinical Neurophysiology, Glostrup Hospital, Glostrup, Denmark

Evoked neural activity correlates strongly with rises in cerebral metabolic rate of oxygen (CMRO2) and cerebral blood flow. The speed and magnitude of activity-dependent rises in CMRO2 fluctuates with ATP turnover due to ion pumping. In vitro studies suggest that increases in cytosolic Ca2+ stimulate oxidative metabolism via mitochondrial signaling, but whether this occurs in intact nerve networks is unknown. Here we applied a pharmacological approach to dissect the effects of ionic currents and cytosolic Ca2+ rises of neuronal origin on activity-dependent rises in CMRO2. We used two-photon microscopy and current source density analysis to study real-time Ca2+ dynamics and transmembrane ionic currents in relation to CMRO2 in the mouse cerebella cortex in vivo. We report a direct correlation between CMRO2 and the summed field excitatory postsynaptic currents (ΣfEPSC) in Purkinje cells (PCs) evoked by stimulation of the climbing fibers. Blocking stimulus-evoked rises in cytosolic Ca2+ in PCs with the P/Q-type channel blocker w-agatoxin-IVA (ω-AGA), or the GABAA receptor agonist muscimol, did not reduce CMRO2, excitatory synaptic or action potential currents in a time-locked manner. During stimulation, neither ω-AGA or Ru360, a mitochondrial Ca2+ uniporter inhibitor, affected the ratio of CMRO2 to fEPSCs or evoked field potentials (LFPs). However, baseline
CBF and CMRO2 decreased gradually with Ru360. Our data suggest that in vivo activity-dependent rises in CMRO2 are correlated with synaptic currents and postsynaptic spiking in PCs. Our study could not confirm a unique role of neuronal cytosolic Ca2+ signals in controlling CMRO2 increases during climbing fiber stimulation in mice.
Mitochondrial autophagy, or mitophagy, is a crucial protective response whose dysregulation can lead to buildup of dysfunctional mitochondria and cell death. Its regulation in neurons is largely unknown, as much of the work on mitophagy has been done in yeast and non-neuronal cell lines. Recently, defects in mitophagy have been implicated in neurodegenerative diseases such as Parkinson’s disease and Huntington’s disease. However, current methods to assay mitophagy in neurons are limited by their inability to track defined populations of mitochondria over extended periods of time in live cells and can only provide snapshots of mitochondrial flux. We sought to develop a sensitive and quantitative technique to monitor the autophagy-mediated flux of mitochondria in live neurons. By photoconverting a subset of a mitochondrially targeted fluorescent protein, mito-EOS2, and following this photoconverted mitochondrial population over time with automated microscopy, we can use the rate of decay of the photoconverted mitoEOS2 fluorescence as a measure of mitochondrial degradation by mitophagy. This rate is sensitive to pharmacological inducers of mitophagy both in neuronal cell lines and primary neurons and can be inhibited by knockdown of crucial autophagy proteins. Finally, we show that the rate of mitophagy is modulated by mutant huntingtin expression in a primary neuron model of Huntington’s disease.
Acetyl-L-carnitine protects mitochondrial enzymes and improves long-term outcome after neonatal hypoxic-ischemic brain injury

Mary C. McKenna¹,²,³, Jaylyn Waddell¹, Michele Diamond¹, Timothy Kelly¹, Dorothy Demers¹, Da Shi¹,³, Susanna Scafidi¹

University of Maryland School of Medicine, 1 Department of Pediatrics, 2 Program in Neuroscience and 3 Graduate Program in Life Sciences, Baltimore, Maryland, USA 21201

Neonatal hypoxia-ischemia (H/I) occurs at a rate of ~ 3/1000 live births in the US and is a common cause of neurological injury in infants. We used the Vannucci method of H/I in 7 day old rat pups (ligation of right carotid artery followed by 85 min of hypoxia) and determined acute alterations to mitochondrial enzymes and proteins (0.25-72 hr), and long term effects on brain metabolism, neurotransmitter synthesis and novel object recognition 3-4 weeks after injury. Some rat pups were treated with acetyl-L-carnitine (ALCAR) at 0, 4, 24 and 48 hr after H/I. The impaired mitochondrial respiration previously reported after H/I could be due, in part, to alterations in key mitochondrial enzymes including the pyruvate dehydrogenase complex (PDH), α-ketoglutarate dehydrogenase (KGDH), and/or other mitochondrial proteins. Protection of PDH activity after injury allows the pyruvate from glucose metabolism to undergo oxidative metabolism in the TCA cycle. KGDH is important since it is a TCA cycle enzyme that oxidizes the α-ketoglutarate formed from glutamate, and thus has a key role in protecting the developing brain from excitotoxic injury. H/I injury led to an acute decrease in PDH activity at 2-4 hr after injury and a delayed decrease in KGDH activity in H/I side of brain at 48 hr and 8 days after H/I compared to enzyme activity in controls. Rat pups treated with ALCAR after H/I had PDH and KGDH activity comparable to controls. Treatment of rat pups with ALCAR
after Injury led to improved memory as assessed by the novel object recognition test. At 24 hr after initial testing the H/I rat pups showed no preference for a novel object demonstrating that they did not remember the objects. In contrast, the control and ALCAR treated rats showed a preference for the novel object indicating that they retained their memory of the objects from the previous day of testing. Overall our data show that H/I lead to both acute and delayed impairment of mitochondrial enzymes early after injury and long-term impairment of oxidative energy metabolism, neurotransmitter synthesis and memory. Our studies show that treatment with ALCAR after H/I injury protected both PDH and KGDH activity and led to long term improvement in memory as determined by novel object recognition. Our studies underscore the potential of treatment with ALCAR as a safe, effective therapy for protecting the developing brain from injury.

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Pharmacological activation of the PPAR/PGC-1α pathways has beneficial effects on the development and progression of a mitochondrial encephalopathy

Natalie Noe¹, Veronika Lellek¹, Francisca Diaz², Aline Hida², Carlos T. Moraes²,³ and Tina Wenz¹

¹ Institute for Genetics and Cluster of Excellence: Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany; ² Department of Neurology and ³ Department of Cell Biology and Anatomy, University of Miami, School of Medicine, Miami, FL, USA

Mitochondria play a vital role in the maintenance of neuronal function and mitochondrial dysfunction is closely linked to neurodegenerative diseases. While the link between mitochondrial dysfunction and neurodegeneration is not clear, the organelle appears to be the site for initiation and propagation of disease processes. We could previously show that activation of the PPAR/PGC-1α pathway preserves mitochondrial and tissue function in skeletal muscle during disease and aging. We now investigated the activation of this pathway on mitochondrial function and its link to processes in the central nervous system, that drive neurodegeneration. We used a mouse model with a defect in mitochondrial function which exhibits astrogliosis and neuronal atrophy. We induced the PPAR/PGC-1α pathway by pharmacological stimulation with bezafibrate. We could show that bezafibrate-administration markedly improved the phenotype of the mice: Loss of motoric function was largely attenuated. Additionally, brain atrophy was slowed down. We found that PGC-1α levels were upregulated in cortex and hippocampus upon bezafibrate treatment indicating that the drug passes the blood brain barrier. Accordingly, we saw increase in mitochondrial proteins and mitochondrial ATP generating capacity in bezafibrate treated animals. Interestingly, bezafibrate treatment also attenuated several processes that are linked to neurodegeneration such as
oxidative stress, inflammatory response and apoptosis presumably resulting in the increased neuroprotection in the treated animals. Work in cell culture further underlines this linkage of mitochondrial function and neuronal stress response and the modulation of this linkage by bezafibrate. Although bezafibrate treatment could not completely ameliorate the neuronal loss and the resulting brain atrophy, our results show that bezafibrate has widespread beneficial effect on several aspects that are involved in the pathogenesis of a mitochondrial encephalopathy.
Direct mapping of FDG uptake in situ at subcellular resolution using soft X-ray fluorescence

Carole Poitry-Yamate¹, Alessandra Gianoncelli², Burkhard Kaulich², George Kourousias², and Rolf Gruetter¹,³,⁴

1 Institute of Physics of Biological Systems – Center for Biomedical Imaging (CIBM) Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
2 ELETTRA – Sincrotrone Trieste S.C.p.A., Trieste-Basovizza, Italy
3 Department of Radiology, University of Lausanne, Lausanne, Switzerland
4 Department of Radiology, University of Geneva, Geneva, Switzerland

Glucose metabolism is essential to brain function, yet its cell-specificity has not yet been completely established due to the difficulty to image with cellular resolution and thereby to distinguish neurons and glia. We therefore exploited synchrotron-based low energy X-ray fluorescence (LEXRF) imaging of low-atomic number Z elements, carbon, oxygen, nitrogen and fluorine in 3-µm thick slices. Unlike the ¹⁴C/³H/¹⁸F autoradiographic deoxyglucose technique, the LEXRF signal of F is based on its unique x-ray energy of 676 eV, excited in a focal spot of ~1 micron diameter. After fluorodeoxyglucose (FDG) administration glucose utilization was assessed from the fluorine LEXRF signal of intracellular FDG6P at 1µm spatial resolution. The ability to map and spectrally quantitate FDG uptake was validated in retinal preparations, reproducing the localization of glucose metabolism to glial Müller cells. Similarly, in hypothalamus, a high uptake of FDG into glial tanycytes was observed. However, in ventromedial hypothalamus and motor cortex, FDG uptake was high in neuropil, and detected in putative endoplasmic reticulum within neuronal cell bodies, showing directly for the first time that neurons also metabolize glucose. We conclude that differential glucose uptake into glia and neurons is region-dependent, supporting the conventional view that glucose is the principal metabolic
substrate for glia and neurons in structures resembling the general cerebral cytoarchitecture of mature mammalian brain. Therefore, the FDG PET signal likely reflects both neuronal and glial metabolism. Coupled to the subcellular resolution capabilities and the quantitative nature of elemental detection, we further conclude that synchrotron based LEXRF combined with scanning transmission x-ray micrography (STXM) together hold remarkable potential for assessing FDG metabolism by brain cells.
Oxidative stress, mitochondrial dysfunction and energetic stress are common features in acute and chronic neurodegenerative disorders. However, little remains known about the exact metabolic and molecular underpinnings and even less about how neuronal metabolism can be targeted to provide neuroprotection. We addressed these questions by studying glutamate excitotoxicity in cultured cortical neurons. Upon excessive glutamate-receptor stimulation, calcium overload will trigger a vicious circle of oxygen radical formation, mitochondrial dysfunction and ATP depletion, ending up in neuronal death. We showed that cortical neurons deficient for PHD1 are largely protected against excitotoxic death. PHD1 (Prolyl Hydroxylase Domain Protein 1) belongs to family of oxygen sensors, orchestrating an adaptive response against hypoxic and oxidative stress. We previously documented that absence of PHD1 provided hypoxia tolerance to muscle by reprogramming metabolism from oxidative to glycolytic utilization of glucose, thereby reducing oxygen consumption. Surprisingly, however, PHD1 deficient neurons showed a reduction in glycolytic flux rate and glucose oxidation. Interestingly, the oxidative pentose-phosphate pathway (PPP) was substantially induced. The PPP represents an alternative route for glucose-6-phosphate,
critical for redox homeostasis by generating NADPH, necessary to replenish reduced glutathione. Indeed, PHD1 deficient neurons had lower superoxide levels upon an excitotoxic stimulus. TIGAR is a recently discovered gene that directs the fate of glucose-6-phosphate into the PPP, presumably by reducing the activity of PFK1, a rate-limiting enzyme of glycolysis. PHD1 deficient neurons induced the expression of TIGAR, which is the likely mediator of the metabolic shift from glycolysis towards the PPP that lowers ROS levels. Since a reduction in glucose oxidation could potentially threaten energy homeostasis, we investigated the oxidation of alternative mitochondrial substrates. Indeed, the oxidation of glutamine was enhanced in PHD1 deficient neurons. In this project we propose how PHD1 deficiency alters glucose and glutamine metabolism to provide redox and energy homeostasis in excitotoxic conditions. We are currently exploring possible novel therapeutic avenues for neurological diseases that implicate oxidative stress and glutamate excitotoxicity.
Acetate: compartmentation, synthesis and metabolism in cortical tissue slices

Caroline Rae¹, Aurelie Fekete¹, Mohammed Kashem¹, Fatima A Nasrallah¹ & Stefan Bröer²

¹ Neuroscience Research Australia & UNSW, Sydney, Australia; ² Research School of Biology, The Australian National University, Canberra, Australia

Acetate is a two carbon intermediate in metabolism. It has long been accepted as a marker of astrocytic metabolism, and a substrate for production of metabolites such as glutamine, glutamate and citrate. Using [3-13C]pyruvate, [3-13C]lactate and/or [1-13C]glucose as a substrate for Guinea pig brain cortical tissue slices, we describe production of [2-13C]acetate by slices under a range of experimental conditions. We have measured the KM for acetate for the high affinity (neuronal) lactate transporter MCT2 (2.58 ± 0.8 mM) which indicates that, at the concentration of acetate usually used (5 mM), acetate should be taken up by neurons. We conducted experiments competing labeled and unlabelled substrates with one another to examine compartmentation of monocarboxylate metabolism. From these we conclude: acetate is taken up by brain slices to a concentration in excess of that in the medium, suggesting active uptake, possibly via a sodium-dependent monocarboxylate transporter. Only a small (< 9%) of this acetate is metabolized via the Krebs cycle, with other substrates such as pyruvate and lactate being used in preference. Acetate is metabolized in a small glial compartment which likely represents a fraction of glial metabolism. Acetate interacts with citrate production in astrocytes but does not contribute significantly to it. Acetate is produced from acetylCo-A in a compartment identical to that labeled with low (0.74 mM) concentrations of lactate, suggesting that neurons may produce acetate. Addition of acetate stimulated production of acetate, and also stimulated production of citrate from another, possibly astrocytic
compartment. It would appear as though the steady-state equilibria in cortical brain slices are arranged so as to minimise brain acetate catabolism. Given the roles of acetate units in synthesis of the neurotransmitter acetylcholine, as well as an important constituent of myelin it may be counterproductive for brain to actively and easily catabolise acetate.
Mitochondrial bioenergetics, cellular energy transfer and Na+,K+-ATPase are compromised by 3-methylcrotonylglycine in brain of young rats


Dept. of Biochemistry, Univ. Federal do Rio Grande do Sul, Porto Alegre, Brazil

3-Methylcrotonyl-CoA carboxylase deficiency (3-MCCD) is an inherited metabolic disorder biochemically characterized by high tissue accumulation and urinary excretion of 3-hydroxyisovalerate, 3-methylcrotonylglycine (3-MCG) and 3-hydroxyisovaleryl-carnitine. Clinically, patients present severe brain abnormalities and neurologic dysfunction, whose pathomechanisms are still unclear. In the present study we investigated the in vitro effects of 3-MCG (0.1 -5mM) on important parameters of bioenergetics and Na+, K+-ATPase in cerebral cortex of 30-day-old Wistar rats. Rats were sacrificed by decapitation, the cerebral cortex isolated, homogenized and used for the biochemical assays. 3MCG significantly reduced CO2 production from acetate (30%) and the activity of complex II-III (35%). 3MCG also inhibited the activities of mitochondrial creatine kinase (mCK) (65%) and Na+, K+-ATPase (45%). Furthermore, antioxidants attenuated or fully prevented the effect of 3MCG on the activities of mCK and Na+, K+-ATPase, suggesting the involvement of reactive species on these inhibitory effects. In conclusion, our results demonstrate that 3-MCG impairs brain bioenergetics at the level of energy formation, transfer and utilization. Therefore, it is presumed that these mechanisms may be involved in the pathophysiology of the neurological dysfunction that occurs in patients affected by 3-MCCD.
Financial support: research grants from CNPq, PROPESq/UFRGS, FAPERGS, PRONEX, FINEP Rede Instituto Brasileiro de Neurociência (IBN-Net), INCT-EN, Programa Nacional de Pós-Doutorado - CAPES.
Assessment of brain energetics at different life stages in the Brown Norway rat

Joyce E. Royland¹, Prasada Rao S. Kodavanti²
and Robert C. MacPhail²

1 Integrated Systems Toxicology and 2 Toxicology Assessment Divisions, NHEERL/ORD, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA.

A major concern in assessing toxicity to environmental exposures is differential susceptibility in the population. Aging adults comprise the fastest growing segment of the population and may possess a greater sensitivity due to changes in metabolic, reserve and repair capacities. Loss of mitochondrial integrity is an often cited cause of aging and mitochondrial ROS production is an accepted toxicity pathway. Consequences of mitochondrial dysfunction include decreased energy metabolism, and increased generation of reactive oxygen species potentially resulting in loss of neuronal function and plasticity and, ultimately, cell death. As part of our goal to identify key biochemical pathways that contribute to increased susceptibility, we studied processes related to function or loss of function in the mitochondria. Initially we examined gene expression in pooled cerebellar samples from 4 and 24 month old male Brown Norway rats using commercial RT-PCR oxidative stress and neurogenesis arrays as measures of mitochondrial dysfunction and neural repair, respectively. That study showed the majority of oxidative stress-related genes were up-regulated (> 1.25 fold) while genes related to neurite growth/repair were predominantly down-regulated (> 1.25 fold). In a follow-up study, gene expression in cerebellum, striatum, hippocampus and frontal cortex from 4, 12 and 24 month old Brown Norway rats was examined using a custom designed RT-PCR array containing 30 genes representing processes associated with mitochondrial function (energy metabolism, ROS generation), neuronal maintenance (plasticity) or cellular response to damage (inflammation and stress responses). All
processes measured were impacted, with number and direction of individual gene expression changes varying with brain area. Both quantity and magnitude of gene expression changes tended to increase with age. In general, genes related to energy metabolism were down regulated, as were those related to plasticity and response to stress or oxidative damage. Immune/inflammation responsive genes were the most affected and showed large age-related increases in expression in every brain area (up to 18 fold in the striatum). The hippocampus was most sensitive to aging based on more genes affected and earlier changes in expression (i.e. by 12 months of age). However, based on level of expression changes, the striatum was most vulnerable. These data provide further evidence of age-related loss of mitochondrial function with resultant adverse consequences as indicated by markers of inflammation. In addition, we see evidence of declines in the capacity of the brain to withstand insult (i.e. plasticity) such as might occur with environmental exposures. (This abstract does not necessarily reflect USEPA policy).
Glycogen is crucial for astrocytes and neurons in brain function

Isabel Saez\textsuperscript{1,2}, Jordi Duran\textsuperscript{1,3}, Flor Tevy\textsuperscript{1}, Agnes Gruart\textsuperscript{4}, Jose Maria Delgado- García\textsuperscript{4} and Joan Guinovart\textsuperscript{1,2,3}

1 Institute for Research in Biomedicine, IRB Barcelona, Barcelona, Spain
2 Department of Biochemistry and Molecular Biology, University of Barcelona, Barcelona, Spain
3 Centro de Investigación Biomédica en Red de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Madrid, Spain
4 Division of Neurosciences, Pablo de Olavide University, Seville, Spain

It has been proposed that brain glycogen is essential for neuronal functioning during situations of high energy demand, such as hypoglycemia, hypoxia/ischemia or even memory consolidation. In this study we sought to further characterize the biological function of glycogen in brain. For this purpose, by deleting Glycogen Synthase (GS) specifically in the nervous system, we generated a genetically modified mouse characterized by the absence of glycogen in brain. We observed that these animals present a diminished learning capacity, as assessed by the Skinner Operant Conditioning Test, as well as impairment in the Long-Term Potentiation. These results confirm the relevance of brain glycogen in the process of memory acquisition.

Although brain glycogen is localized mainly in astrocytes, neurons have the necessary machinery to synthesize this polysaccharide. However, its biological significance in the brain has not been addressed. Here we worked with pure neuronal cultures that were exposed to hypoxic conditions. We show that neurons have a basal glycogen pool, which is degraded under oxygen deprivation. To address the importance of this glycogen, we analyzed the mortality of neurons from GS knock out mice in hypoxia. These neurons registered a higher mortality rate than those from the wild-type mice. To reinforce the relevance of neuronal glycogen in another in vivo system, we generated a Drosophila
Melanogaster model, which has reduced levels of GS in neurons but not in astrocytes. When exposed to a low oxygen environment, these flies presented an impaired behavioral response.

In conclusion, our results highlight the importance of glycogen in brain and indicate that, contrary to what has been widely accepted, neurons have a basal glycogen pool, which is required in stress situations, such as hypoxia.
Lipoic acid as a therapeutic/nutraceutical Agent to treat early stages of Alzheimer’s disease

Harsh Sancheti, Enrique Cadenas

Department of Molecular Pharmacology and Toxicology, School of Pharmacy
University of Southern California, Los Angeles, CA 90089

Neuronal energy, critical for the brain to function correctly, is mainly supplied by glucose. Human brain has the highest consumption of glucose with respect to its size and longitudinal studies have shown that decreased brain glucose uptake far precedes the pathology associated with AD. A bioenergetic deficit precedes the impairment of synaptic plasticity, widely considered to be strongly associated with learning and memory, two components that are extensively deregulated in AD. AD symptoms usually develop slowly and worsen over time, becoming severe enough to interfere with daily tasks and disrupt learning and memory-dependent activities.

Thus, energy deficit and synaptic plasticity are the main issues to be addressed for preventing the progression of AD. Lipoic acid (LA), a cyclic disulfide, has been shown to increase glucose uptake in several tissues and improve age-related decline in synaptic plasticity. We used the triple transgenic AD mice model (3xTgAD), harboring three transgenes and showing AD associated pathogy i.e., plaques and neurofibrillary tangles in an age dependent manner, to study the effects of LA feeding.

Our data show that the 6 month old 3xTgAD mice showed significantly less total brain glucose uptake, measured by PET-CT imaging, in comparison with the wild-type mice, and LA feeding to these mice for one month could partially recover the decreased glucose uptake. The 3xTg-AD mice showed decreased Akt phosphorylation and downstream PI3K/Akt signaling in comparison with the wild-type mice. Akt
phosphorylation and PI3K/Akt signaling could be substantially restored by feeding LA. Moreover, the central energy-regulation pathway i.e., AMPK was also less activated in 3xTg-AD mice compared to wild-type, and it could be significantly increased by LA feeding. Overall, our data show that the insulin-like effect of LA restores PI3K/Akt and AMPK signaling, and stimulates mitochondrial bioenergetics, thus overcoming the energy deficit inherent in AD.
**Malic enzyme can support neuronal superoxide production by providing NADPH**

Reno Reyes¹, Ji-Eun Kim¹, Angela M. Brennan¹, Raymond A. Swanson¹

1 Depts. of Neurology, San Francisco Veterans Affairs Medical Center and University of California, San Francisco, U.S.A.

Prior studies show that glutamate excitotoxicity involves activation of NMDA receptors and resultant neuronal production of superoxide. Inhibition of neuronal NADPH oxidase (NOX2) prevents glutamate-induced superoxide production and prevents excitotoxic neuronal death. NOX2 utilizes cytosolic NADPH to produce superoxide (O2⁻) from O2. One source of cytosolic NADPH is the hexose monophosphate shunt, which uses glucose as a substrate. Prior studies have shown that neurons maintained with energy substrates other than glucose are unable to generate superoxide during NMDA receptor activation. If this effect of glucose deprivation is in fact due to reduced NADPH production, then this effect should be negated when neurons have an alternative means of generating NADPH. An alternative source of NADPH production is malic enzyme, which catalyzes the reaction: malate + NADP+ ⇌ pyruvate + CO2 + NADPH. Here we supplied neurons with malate to see if this would reconstitute superoxide formation in neurons exposed to NMDA under glucose-free conditions. Superoxide formation was measured by both the dihydroethidium method and by formation of 4-hydroxynonenal. Treatment groups and results were as follows:

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Malate was found to reverse the effects of glucose deprivation on NMDA-induced superoxide production and neuronal death (arrows). These results suggest a novel link between neuronal metabolism and excitotoxic injury.
Astrocytic glutamate uptake are responsible by prevention of excitotoxicity in brain. Recent evidences suggest that the fate of uptaken glutamate depends of the amount of sodium efflux by Na+/K+ ATPase in astrocytes, being glutamate uptake the major contributor for this. Increased influx of glutamate in astrocytes favors its conversion to alpha-ketoglutarate and flux to tricaboxylic acid cycle/ oxidation to CO2 instead of conversion to glutamine. In order to assess the importance of glutamate as brain energetic substrate and its effect upon glucose utilization in excitotoxic conditions, hippocampi isolated from adult Swiss albine mice were incubated in Dulbecco’s medium pH 7.3 with 5.0 mM D-glucose, containing either (i) 0.1 mM L-glutamic acid ± 10 μM N-methyl-D-aspartate (NMDA) or (ii) 1.0 mM L-glutamic acid ± 10 μM NMDA for 30, 60 and 120 minutes. CO2 production were measured using radio-labeled substrates, D-[U-14C] glucose or L-[U-14C] glutamate. Data are presented as mean and SEM in pmol of substrate/mg of tissue. Statistical analysis used two-way ANOVA followed by simple main effect test (SMET) if detected interaction. Presence of 10 μM NMDA increased glutamate oxidation at 120 min in media with 100 μM glutamate (glutamate 100 μM: 136.1 ± 7.9 / 272.6 ± 6.7 / 652.7 ± 55.9; glutamate 100 μM + NMDA 10 μM: 123.3 ± 6.0 / 332.5 ± 21.9 / 792.3 ± 27.2 - for 30, 60 and 120 min respectively). In media with 1 mM glutamate, NMDA impaired glucose oxidation at 30 min (glutamate 1 mM: 945.7 ± 28.6 / 1471 ± 61.3 / 3127 ± 96.1; glutamate 1 mM + NMDA 10 μM: 509.2 ± 31.3 / 1576 ± 136.7 / 3267 ± 194.8).
glutamate 1 mM caused 4 to 7-fold increase in glutamate oxidation with or without concomitant NMDA. Presence of NMDA increased glucose oxidation for all times studied, either in media with glutamate 100 μM (glutamate 100 μM: 414 ± 20.7 / 821.7 ± 43.2 / 2298 ± 104.1; glutamate 100 μM + NMDA 10 μM: 509.8 ± 19.1 / 1071 ± 67.3 / 2373 ± 113.2) or 1 mM (glutamate 1 mM:420.6 ± 12.6 / 620.5 ± 62.2 / 1783 ± 177.7; glutamate 1 mM + NMDA 10 μM: 520.3 ± 29.9 / 927.7 ± 42.6 / 2048 ± 74.5).

Note that presence of glutamate 1 mM decreased glucose oxidation after 120 minutes of incubation with or without presence of NMDA. Sustained increase of extracellular glutamate concentration drives its metabolic fate to TCA cycle. In the other hand, our data suggests lower glucose oxidation for longer periods of incubation under these conditions. The preference of glutamate (higher oxidation to CO2 than glucose) as oxidative substrate in the excitotoxic condition could suggest that some of the oxidized glutamate could are being converted to lactate in astrocytes and shuttled to neurons.
In vivo assessment of brain mitochondrial coupling using NMR spectroscopy

Julien Valette, Myriam Chaumeil, Emmanuel Brouillet and Vincent Lebon

1 Commissariat à l’Energie Atomique (CEA), Institut d’Imagerie Biomédicale (I2BM), Molecular Imaging Research Center (MIRCen), 18 route du panorama, 92260 Fontenay-aux-Roses, France

An important parameter to characterize the efficiency of the oxidative phosphorylation system, i.e. mitochondrial coupling, is the (P/O) ratio, which represents the number of ATP molecules synthesized per oxygen consumed. Regulation of the (P/O) ratio is likely an important aspect of brain physiology. In addition, alterations in (P/O) ratio homeostasis may play a key role in neurodegenerative diseases, such as Huntington’s disease.

Regional measurements of (P/O) ratio in vivo remain extremely challenging. While various methods based on positron emission tomography (PET) or magnetic resonance spectroscopy (MRS) exist to assess substrate consumption and oxidation, the only direct and non-invasive method to measure the rate of ATP synthesis in vivo is 31P-MRS. One major difficulty with this method is to efficiently localize signal without causing signal destruction (due to very short transverse relaxation times T2).

Here we present a multimodal approach that allowed regional measurements of (P/O) ratio in the monkey brain. First, cerebral metabolic rate of glucose (CMRglc) was measured by 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography (PET). Tricarboxylic acid (TCA) cycle rate (VTCA) was measured with an indirect 13C-MRS approach developed in-house, following infusion of uniformly labeled 13C-glucose. Finally, ATP synthesis rate (VATP) was measured using 31P-MRS combined with a progressive saturation transfer approach, where signal localization was achieved.
with an original outer volume suppression strategy, minimizing signal loss due to relaxation. All measurements were performed in an 8-mL voxel positioned in the cortex and striatum in three male macaques, using an ECAT EXACT HR+ Siemens tomography for PET and a whole-body 3 teslas Bruker scanner for MRS. Measured flux values were CMRglc=0.27±07 µmol/g/min, VTCA =0.63±0.12 µmol/g/min, and VATP=7.8±2.3 µmol/g/min. Measured VTCA/CMRglc appears to be ~2, indicating that glucose is the main fuel for TCA cycle, in the healthy brain in primates. More interestingly, since stoichiometry imposes that oxygen consumption VO=6VTCA, we can estimate (P/O)=VATP/VO~2. This is very close to the maximal theoretical (P/O) ratio, which is ~2.2 as calculated when assuming fully efficient mitochondrial coupling. Thus the in vivo brain P/O ratio we determined indicates minimal brain mitochondrial uncoupling in physiological conditions.

In conclusion, the present multimodal approach is the first report of localized (P/O) measurement in the primate brain in vivo. This strategy should be of interest to investigate the role of mitochondrial coupling in models of neurodegenerative diseases.
Foundations of Alzheimer’s bioenergetic vulnerability in young adult APOE ε4 carriers

Andrew B. Wolf¹, Nikki Konkowski², Luke Henderson¹, Randall Allred¹, Jon Valla¹,³

¹ Midwestern University, Glendale, AZ, USA; ² University of Arizona, Phoenix, AZ, USA; ³ Arizona Alzheimer’s Consortium, Phoenix, AZ, USA

Individuals carrying the APOE ε4 allele are at dramatically elevated risk for developing late-onset Alzheimer’s disease (AD). Previous imaging studies using FDG PET showed that young-adult (age 18-39) carriers of APOE ε4 display a regional pattern of reduced glucose metabolism similar to that apparent in AD patients and aged APOE ε4 carriers. The posterior cingulate cortex, a hub in the resting-state default mode network, displays particularly significant metabolic reductions. Our work applying cytochrome oxidase histochemistry in postmortem tissues from AD patients and young-adult APOE ε4 carriers revealed that both also demonstrate significant reductions in brain neocortical mitochondrial functional activity, and in the same regional (i.e., posterior cingulate) and laminar pattern of vulnerability (i.e., superficial lamina most effected). Interestingly, in the young-adults, these reductions occur in the absence of apparent AD-related neuropathology. We believe this finding represents the earliest AD-related functional brain alteration yet described in this at-risk population, and that it indicates that bioenergetics may contribute significantly to how APOE is involved in AD. It is not known what mechanism confers this potential bioenergetic vulnerability, or how deficits in young adults relate to the increased likelihood of developing AD decades later. We have initiated a program to address these two questions directly. We are currently evaluating gene and protein expression and neuronal morphology/synaptic complexity in subsets of the same young-adult samples previously analyzed. Preliminary results indicate a pronounced downregulation of both
mitochondrial- and nuclear-encoded subunits of the electron transport chain (ETC), most prominently of cytochrome oxidase. We are currently working to verify that this represents downregulation and not a defect in ETC function. Further, we have begun to assess neuronal and synaptic morphology toward the hypothesis that APOE ε4 carriers develop less, or alternatively, lose, neocortical dendritic complexity and/or synaptic contacts, leading to the apparent downregulation of bioenergetic pathways in higher-order brain regions.

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Interplay between reactive oxygen species and mitochondrial dysfunction during neuroinflammation

Jamie Lim¹, Maarten Witte², Philip Nijland², Joost Drexhage¹, Susanne van der Pol¹, Elga de Vries¹, Jack van Horssen¹

¹ Dept. of Molecular Cell Biology and Immunology and 2 Pathology, VU University Medical Center, Amsterdam, The Netherlands.

Multiple Sclerosis (MS) is a neurological disorder characterized by immune cell infiltration, demyelination and neurodegeneration. Although the pathogenetic mechanisms underlying MS remain enigmatic evidence is emerging that reactive oxygen species (ROS) induce mitochondrial dysfunction thereby contributing to MS lesion development and progression. The first aim of our study was to identify the cellular source of ROS in the initial stage of MS lesion formation. Hereto, we analyzed the expression of enzymes involved in ROS production in MS brain tissue and showed by immunohistochemistry and western blot analysis that various subunits of the NADPH-oxidase (NOX)1 and -2 complex are significantly upregulated in early MS lesions compared with control white matter. Antibodies directed against NOX1 and -2 subunits predominantly decorated activated microglia and infiltrated macrophages. Based on these findings we postulated that enhanced NOX1 and -2 expression results in increased ROS production, which subsequently induces mitochondrial injury. This will eventually promote ROS formation as dysfunctional mitochondria will generate more ROS resulting in a vicious circle. To coordinate the delicate redox balance brain cells and mitochondria are endowed with an intricate defence system consisting of antioxidant enzymes. We showed that cytoplasmic as well as mitochondrial antioxidant enzymes are markedly upregulated in active MS lesions. It is conceivable that enhanced antioxidant enzyme production during a neuroinflammatory
attack reflects an adaptive defence mechanism to reduce ROS-induced cellular damage. Nonetheless, we and others demonstrated that markers of oxidative damage are abundantly present in active MS lesions indicating that the increase of antioxidant enzymes is insufficient. Importantly, recent clinical studies indicate the therapeutic potential of antioxidant therapy. Here, we studied the distribution of the transcription factor Nrf2, a transcriptional master regulator of antioxidant production and Nrf2-driven antioxidants in MS lesions. Nrf2 and Nrf2-regulated antioxidant enzymes predominantly localized to astrocytes and infiltrated macrophages, not in oligodendrocytes. Therefore, we hypothesized that activation of the Nrf2 pathway might limit ROS-mediated oligodendrocyte injury. Finally, we demonstrated that activation of the Nrf2 pathway induced antioxidant enzyme production in primary oligodendrocytes and rescued oligodendrocytes from an oxidative insult.
Impairment of brain redox and bioenergetics homeostasis caused by the major metabolites accumulating in HHH syndrome

Carolina M. Viegas¹; Alexandre U. Amaral¹; Aelise M. Tonin¹, Estela NB Busanello¹, Moacir Wajner¹

¹ Department of Biochemistry, ICBS-UFRGS, Porto Alegre, RS, Brazil

Tissue accumulation of ornithine (Orn), homocitrulline (Hcit) and ammonia is the biochemical hallmark of patients affected by hyperornithinemia–hyperammonemia–homocitrullinuria (HHH) syndrome, a disorder clinically characterized by neurological symptoms, whose pathophysiology is practically unknown. In the present study we investigated the in vitro and in vivo effects of Orn and Hcit on important parameters of energy metabolism and oxidative stress in cerebral cortex of 30-day-old rats. We verified that Orn and Hcit significantly inhibited in vitro and in vivo the citric acid cycle (inhibition of CO2 synthesis from acetate, as well as aconitase and α-ketoglutarate dehydrogenase activities), the aerobic glycolytic pathway (reduced CO2 production from glucose) and moderately the electron transfer flow (inhibitory effect on complex I–III). We also observed that Orn and Hcit elicited lipid and protein oxidative damage (increased thiobarbituric acid-reactive substances values and carbonyl formation, respectively) and decreased glutathione concentrations in both in vitro and in vivo assays. Furthermore, N-acetylcysteine and the combination of the free radical scavengers ascorbic acid plus α-tocopherol attenuated the lipid oxidation and totally prevented the protein oxidative damage provoked in vivo by Orn and Hcit, suggesting that reactive species were involved in these effects. Hcit in vivo administration, but not Orn, also decreased the activity of catalase and glutathione peroxidase, indicating that Hcit provokes a reduction of brain antioxidant defenses. In conclusion, we postulate that alterations in the cerebral bioenergetics and redox state induced by the major
metabolites accumulated in HHH syndrome may represent pathogenic mechanisms contributing, at least in part, to the neurological symptoms of patients affected by this disorder.
A sub-convulsive dose of kainate compromises astrocytic but not neuronal metabolism in mouse cerebral cortex in vivo

Anne B. Walls\textsuperscript{1} Linn H. Nilsen\textsuperscript{2}, and Elvar Eyjolfsson\textsuperscript{2}, Arne Schousboe\textsuperscript{1}, Ursula Sonnwald\textsuperscript{2}, Helle S. Waagepetersen\textsuperscript{1}

\textsuperscript{1} Dept. of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; \textsuperscript{2} Dept. of Neuroscience, Faculty of Medicine, Norwegian University of Science and Technology (NTNU), Trondheim, Norway

Despite the well-established use of kainate (KA) as a model of seizure activity and temporal lobe epilepsy, most studies have been performed at KA doses leading to general limbic seizures and have focused on neuronal changes. Hence, little is known about the effect of a sub-convulsive dose of KA on cerebral energy and amino acid metabolism and particularly with regard to effects of KA on astrocytic metabolism there is a dearth of data. Thus, astrocytic and neuronal metabolism was investigated in cerebral cortex of adult mice following treatment with saline (controls), a sub-convulsive (3.75 mg/kg) or a mild convulsive (15 mg/kg) dose of KA injected intraperitoneally (i.p.). Cortical metabolism was mapped by concomitant injection of the astrocyte specific substrate \([1,2\,\text{C}]{\text{acetate}}\) plus \([1\,\text{C}]{\text{glucose}}\), which enters both neurons and astrocytes although oxidative metabolism is predominantly a neuronal event. The sub-convulsive dose of KA led to an increase in cortical lactate content and a reduction in the amount of \([4,5\,\text{C}]{\text{glutamine}}\) labeled from \([1,2\,\text{C}]{\text{acetate}}\) while the content of and \text{C} labeling in other metabolites were unaltered compared to control mice. This indicates that the sub-convulsive dose of KA selectively affects astrocytic metabolism. In contrast, the convulsive dose led to decrements in the cortical content of glutamate, glutamine, GABA and aspartate. Moreover, the amounts of \([4,5\,\text{C}]{\text{glutamine}}\) and \([4,5\,\text{C}]{\text{glutamate}}\) labeled from \([1,2\,\text{C}]{\text{acetate}}\) as well as the amounts of \([4\,\text{C}]{\text{glutamate}}\), [4-
13C]glutamine, [2-13C]GABA, [2-13C]aspartate, [3-13C]aspartate, [3-13C]lactate and [3-13C]alanine labeled from [1-13C]glucose were reduced by almost 50% compared to controls. This implies that both astrocytic and neuronal metabolism is affected by the convulsive dose of KA. The results provide evidence for astrocytic metabolism being compromised by a sub-convulsive dose of KA while a higher dose was required to affect neuronal metabolism. Moreover, it appears that KA treatment imposes a biphasic metabolic response where the first phase constitutes anaerobic glycolysis resulting in extensive lactate production followed by a phase with pronounced oxidative metabolism of lactate. These phases are likely determined by oxygen availability dependent upon the rate of blood flow. Finally, despite a reduction in the 13C labeling in glutamine from [1,2-13C]acetate, the 13C labeling in glutamate and GABA was not reduced in mice treated with the sub-convulsive
Parkinson-related LRRK2 mutations impaired mitochondrial dynamics and function in neurons

Xinglong Wang¹, Hisashi Fujioka

Departments of 1 Pathology, 2 Pharmacology, and 5 Neuroscience, Case Western Reserve University, Cleveland, Ohio 44106, USA; 3 Department of Neurology & Institute of Neurology, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China; UTSA Neurosciences Institute and Department of Biology, University of Texas at San Antonio, San Antonio, Texas 78249, USA

Expanding evidences have revealed a prominent role for mitochondrial dysfunction in the pathogenesis of Parkinson’s disease (PD). Mitochondria are dynamics organelles that undergo continual fission and fusion events which serve crucial physiological function. Recent studies demonstrated that the neuronal dysfunction/degeneration in PD was likely due to unbalanced mitochondrial fission and fusion dynamics. Pathogenic mutations in leucine-rich repeat kinase 2 (LRRK2, also known as PARK8) lead to autosomal-dominant PD. Here we demonstrated that wild type LRRK2 overexpression in neuronal cells induced mitochondrial fragmentation, mitochondrial dysfunction and neuronal toxicity which was further exacerbated by overexpression of PD-associated mutants (R1441C or G2019S). Interestingly, LRRK2 was found to physically interact with the key mitochondrial fission protein, DLP1 (also named Drp1), and the LRRK2/DLP1 interaction was enhanced by PD-associated mutations that probably facilitated mitochondrial localization of DLP1. The LRRK2-induced mitochondrial fragmentation, mitochondrial dysfunction and neuronal toxicity could be efficiently blocked by co-expression of dominant-negative DLP1 K38A or mitochondrial fusion protein Mfn2. As GTP-binding deficient mutant LRRK2 K1347A or kinase-dead mutant D1994A had minimal interaction with DLP1, did not increase the mitochondrial DLP1 level and did not impair mitochondrial dynamics/function, we concluded that PD-associated mutant LRRK2 causes impaired regulation of mitochondrial dynamics.
through interaction with DLP1 which causes mitochondrial dysfunction and adversely affects neuronal function in PD, and LRRK2 kinase activity plays a critical role in this process.
Utilization of bioinformatics to identify polyphosphate-related proteins in higher eukaryotes

Zheng WEI, Greg Vatcher and Albert Cheung Hoi Yu*

Neuroscience Research Institute, Peking University; Key Laboratory of Neuroscience (Peking University), Ministry of Education; Key Laboratory of Neuroscience (Peking University), Ministry of Health; Department of Neurobiology, School of Basic Medical Sciences, Peking University, Beijing, China
*Corresponding author
Email: achy@bjmu.edu.cn

Phosphate is one of the fundamental elements of life and is important in all major life processes, especially energy metabolism and signal transduction. Polyphosphate (polyP) is investigated for its essential role as a high-energy phosphate source and a regulatory factor in responses to cellular energy downshifts. Targeting polyP can help to find a solution against diseases with energy metabolism defects. Besides, as a component of the mitochondrial permeability transition pore (mPTP) and an active factor in relation to apoptosis, polyP is contributing to anti-aging studies. However, polyP-synthesizing enzymes (PPKs) and polyP-related proteins have not been fully characterized in higher organisms. In this study, we computationally predicted the binding potentials of polyP-related proteins. We found that the partial structural analogues of PPKs contained important feature information, which were identified through the precomputed structural neighborhoods provided by the DALI server. Then bioinformatics approaches including gene ontology enrichment, sequence motif-based searches, molecular docking calculations and surface charge analysis were applied. The features we found were used to computationally identify polyP-related proteins. Among the features, we reported a significant feature structure — a positively-charged semi-tunnel (PCST) which may be involved in the binding and modification of polyP. Our strategy identified potential polyP-related proteins in higher organisms, providing significant insights for the further exploration of the
physiological roles of polyP and its involvement in pathogenesis. [Supported by National Basic Research Program of China (973 program, 2011CB504400), National Natural Science Foundation of China (30870818, 31070974, 31171009) and Beijing Natural Science Foundation (7091004)]
Reduced expression of PGC-1α underlies mitochondrial changes in multiple sclerosis cortex

Maarten E. Witte¹, C Philip G. Nijland¹, Joost A.R. Drexhage², Wouter Gerritsen¹, Dirk Geerts³, Bert van het Hof², Arie Reijerkerk², Helga E. de Vries², Paul van der Valk¹ and Jack van Horssen²

1 Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands; 2 Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, The Netherlands; 3 Department of Pediatric Oncology/Hematology, Sophia Children's Hospital, Erasmus University Medical Center, Rotterdam, the Netherlands

Neurodegeneration in multiple sclerosis (MS) is now considered the major cause of irreversible clinical disability; however, the mechanisms underlying neuronal injury in MS are poorly understood. Currently, there is a growing body of evidence that points towards mitochondrial dysfunction and associated reactive oxygen species (ROS) formation as important contributors to neurodegenerative processes in MS. Decreased expression and activity of several subunits of the oxidative phosphorylation chain (OxPhos) have been observed in myelinated MS cortex. Here we postulate that alterations in transcriptional regulators of OxPhos subunits may underlie mitochondrial dysfunction in MS grey matter. Gene and protein analysis revealed that levels of PPARγ co-activator-1α (PGC-1α), a transcriptional regulator of many OxPhos genes, were consistently and significantly decreased in non-demyelinated MS cortex in the absence of neuronal loss. Besides regulating OxPhos subunit expression, PGC-1α controls expression of mitochondrial specific antioxidants (superoxide dismutase 2, peroxiredoxin 3 & thioredoxin 2), which detoxify ROS, and uncoupling proteins (UCP2, 4 & 5), which reduce mitochondrial ROS production. Therefore, we next analyzed expression of these proteins, which together are crucial for safeguarding the mitochondrial redox balance, in our cortical samples. Gene and protein analysis revealed
that there was an overall reduction in mitochondrial antioxidants and UCP4 and 5 expression per mitochondrion in MS cortex. Immunohistochemical analysis revealed that PGC-1α, mitochondrial antioxidants and UCP4 and 5 are predominantly expressed in neurons, indicating a specific loss of neuronal protein expression. Silencing of PGC-1α gene transcription by short hairpin RNA markedly reduced the levels of mitochondrial antioxidants, UCPs and OxPhos subunits in a neuronal cell line. Moreover, PGC-1α silencing resulted in decreased mitochondrial membrane potential, increased ROS formation and enhanced susceptibility to ROS-induced cell death, indicating that PGC-1α is crucial in controlling mitochondrial function. Taken together, our data indicate that reduced PGC-1α expression in MS cortex may underlie the previously observed decrease in OxPhos activity and renders neurons more vulnerable to the oxidative environment thereby contributing to neurodegeneration in MS.
Shift in brain mitochondrial bioenergetics in aging and Alzheimer’s disease: Implications for disease prevention and treatment

Jia Yao¹, Fan Ding¹, Shuhua Chen¹, Jennifer Mao¹, Enrique Cadenas¹, and Roberta Diaz Brinton¹²

¹ Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA, ² Programs in Neuroscience, University of Southern California, Los Angeles, CA, USA

Both basic science and clinical studies have indicated the critical role of mitochondrial bioenergetics in the pathogenesis of Alzheimer’s disease. Previously, we demonstrated that mitochondrial bioenergetic deficits preceded AD pathology in the female triple transgenic AD (3xTgAD) mouse model. Further, decline in brain glucose metabolism is accompanied with activation of alternative fuel source pathways in 3xTgAD mice at early stage. To characterize the age-/AD- associated brain bioenergetic shift, we conducted functional analyses in female triple transgenic Alzheimer’s mice and age-matched non-transgenic (nonTg) at different age groups. In 3xTgAD female, decline in brain glucose metabolism and mitochondrial bioenergetics is first accompanied by the simultaneous activation of the alternative ketone utilization pathways, which is temporary and progresses into activation of fatty acid oxidation (FAO) pathways with disease progression into advanced stage. As a initial translational endeavor to investigate the therapeutic potential of bioenergetic formulations to rescue mitochondrial bioenergetic deficits and delay the progression of Alzheimer’s pathology, 3xTgAD female mice at 6 month were fed with either regular diet (AIN-93G) or diet containing 0.04% 2-DG for 7 weeks. Both mitochondrial bioenergetic parameters and AD pathological markers were analyzed upon completion of the treatment. In the 3xTgAD female mouse brain, 2-DG diet significantly induced ketogenesis, increased
expression of enzymes involved in ketone utilization and mitochondrial oxidative phosphorylation. More importantly, 2-DG diet induced significant reduction of AD-like amyloid pathology. Mechanistically, 2-DG induced activation of the non-amyloidogenic pathway and increased Aβ clearance. The unique trajectory of alterations in brain metabolic capacity enable a bioenergetic-centric strategy that targets disease-stage specific pattern of brain metabolism for disease prevention and treatment. A combination of a nutraceutical and pharmaceutical intervention that enhances glucose-driven metabolic activity and potentiates mitochondrial bioenergetic function may promote healthy aging and prevent AD. Alternatively during the prodromal incipient phase of AD, sustained activation of ketogenic metabolic pathways coupled with supplement of the alternative fuel source, ketone bodies, could sustain mitochondrial bioenergetic function to prevent or delay further progression of the disease. To achieve optimal therapeutic efficacy, both ketone supplement and activation of ketone utilization pathways are required.
Short-term caloric restriction attenuates age-dependent impairment of mitochondrial function in brain

Fei Yin, Harsh Sancheti, and Enrique Cadenas

Department of Pharmacology and Pharmaceutical Sciences, School of Pharmacy, University of Southern California, Los Angeles, CA, USA

Mitochondrial dysfunction is associated with brain aging and is involved in the development of neurodegenerative diseases. Short-term caloric restriction (sCR) elicits some beneficial effects that are similar to life-long caloric restriction in tissues, such as liver and skeletal muscle. In this study, we characterized the age- and sCR-dependent changes of the mitochondrial energy-redox axis in brain, which includes components involved in mitochondrial bioenergetics and redox homeostasis. Our data show that 2-month of 40% caloric restriction leads to distinct responses in brain of Fischer 344 rats at different ages. Mitochondrial respiration is significantly higher after sCR for rats of 26-month, but declines after sCR for rats of 6-month. Activities of enzymes that control the entry of substrates to TCA cycle, such as pyruvate dehydrogenase (PDH) and succinyl CoA transferase (SCOT), are upregulated by sCR for rats at 26-month. On the other hand, mitochondrial redox components including GSH system and Thioredoxin system show an age-dependent decrease of activities, and after sCR, the activities of these enzymes are upregulated, but in senescent animals only. It was also found that sCR significantly changes mitochondrial redox environment in terms of decreased H2O2 generation and decreased amount of glutathionylated protein for animals at 26-month. Oxidized redox state in mitochondria is also accompanied with decreased NADPH/NADP+ ratio and reduced activity of nicotinamide nucleotide transhydrogenase (NNT), which generates NADPH as reducing equivalents to feed the redox systems. Compared to age-matched control groups, sCR induces higher NNT activity in...
26-month rat brain associated with a higher NADPH/NADP⁺ ratio. In summary, our data show that declined mitochondrial energy metabolism and impaired mitochondrial redox homeostasis by aging are attenuated by 2-month short-term caloric restriction in senescent rats, but the beneficial effects of sCR for young animals are not observed.