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KELLY M., TRUDEL S., BROUILLARD F., BOUILLAUD F., COLAS J., NGUYEN-KHOA T., OLLERO M., EDELMAN A., FRITSCH J.

CFTR inhibitors, CFTRinh-172 and GlyH-101 target mitochondrial functions, independently of chloride channel inhibition.

J. Pharmacol. Exp. Ther., 333 (1), 60-69, 2010

(Services cités : Biochimie Générale, FRE 3210, U845 (AE))

Two highly potent and selective CFTR inhibitors have been identified by high-throughput screening: the thiazolidinone CFTR(inh)-172 and the glycine hydrazide GlyH-101. Inhibition of the CFTR chloride channel by these compounds has been suggested of pharmacological interest in the treatment of secretory diarrheas and polycystic kidney disease. In addition, functional inhibition of CFTR by CFTR(inh)-172 has been proposed to be sufficient to mimic the cystic fibrosis inflammatory profile. In the present study we investigated the effects of the two compounds on ROS production and mitochondrial membrane potential in several cell lines: the CFTR-deficient human lung epithelial IB3-1 (expressing the heterozygous F508del/W1282X mutation), the isogenic CFTR-corrected C38, and in HeLa and A549 as non-CFTR expressing controls. Both inhibitors were able to induce a rapid increase in ROS levels and to depolarize mitochondria in the four cell types suggesting that these effects are independent of CFTR inhibition. In HeLa cells, these events were associated with a decrease in the rate of oxygen consumption, with GlyH-101 demonstrating a higher potency than CFTR(inh)-172. The impact of CFTR inhibitors on inflammatory parameters was also tested in HeLa cells. CFTR(inh)-172, but not GlyH-101, induced nuclear translocation of NF-kappaB. CFTR(inh)-172 slightly decreased IL-8 secretion, whereas GlyH-101 induced a slight increase. These results support the conclusion that CFTR inhibitors may exert non-specific effects regarding ROS production, mitochondrial failure and activation of the NF-kappaB signalling pathway, independently of CFTR inhibition.

LAGOUTTE E., MIMOUN S., ANDRIAMIHAJA M., CHAUMONTET C., BLACHIER F., BOUILLAUD F.

Oxidation of hydrogen sulfide remains a priority in mammalian cells and causes reverse electron transfer in colonocytes.

Biochim. Biophys. Acta - Bioenergetics, 1797 (8), 1500-1511, 2010

(Services cités : FRE 3210)

Sulfide (H₂S) is an inhibitor of mitochondrial cytochrome oxidase comparable to cyanide. In this study, poisoning of cells was observed with sulfide concentrations above 20µM. Sulfide oxidation has been shown to take place in organisms/cells naturally exposed to sulfide. Sulfide is released as a result of metabolism of sulfur containing amino acids. Although in mammals sulfide exposure is not thought to be quantitatively important outside the colonic mucosa, our study shows that a majority of mammalian cells, by means of the mitochondrial sulfide quinone reductase (SQR), avidly consume sulfide as a fuel. The SQR activity was found in mitochondria isolated from mouse kidneys, liver, and heart. We demonstrate the precedence of the SQR over

the mitochondrial complex I. This explains why the oxidation of the mineral substrate sulfide takes precedence over the oxidation of other (carbon-based) mitochondrial substrates. Consequently, if sulfide delivery rate remains lower than the SQR activity, cells maintain a non-toxic sulfide concentration ($<1\text{ }\mu\text{M}$) in their external environment. In the colonocyte cell line HT-29, sulfide oxidation provided the first example of reverse electron transfer in living cells, such a transfer increasing sulfide tolerance. However, SQR activity was not detected in brain mitochondria and neuroblastoma cells. Consequently, the neural tissue would be more sensitive to sulfide poisoning. Our data disclose new constraints concerning the emerging signaling role of sulfide.

2009

BONNEFONT J.P., BASTIN J., BEHIN A., DJOUADI F.

Bezafibrate for an inborn mitochondrial Beta-oxidation defect.

N. Engl. J. Med., 360 (8), 838-840, 2009

(Services cités : FRE 3210, U781)

BOUILLAUD F.

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

Biochim. Biophys. Acta - Bioenergetics, 1787 (5), 377-383, 2009

(Services cités : FRE 3210)

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

CRISCUOLO F., BOUILLAUD F.

Free radicals run in lizard families: a mitochondrial uncoupling phenomenon or not ?

Biol. Lett., 5 (3), 343-344, 2009

(Services cités : FRE 3210)

DJOUADI F., LECARPENTIER Y., HEBERT J.L., CHARRON P., BASTIN J., COIRAULT C.

A potential link between peroxisome proliferator-activated receptor signalling and the pathogenesis of arrhythmogenic right ventricular cardiomyopathy.

Cardiovasc. Res., 84 (1), 83-90, 2009

(Services cités : FRE 3210)

AIMS: Arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterized by major fibro-fatty replacement of the right ventricle (RV). We hypothesized that changes in peroxisome proliferator-activated receptor (PPAR) signalling contributed to myocardium fatty accumulation and contractile dysfunction in ARVC. METHODS AND RESULTS: Real-time quantitative reverse transcriptase-polymerase chain reaction and western blotting were used to assess cardiac expression of PPARalpha and gamma and two of their downstream target genes-medium-chain acyl-CoA dehydrogenase (MCAD) and phosphoenolpyruvate carboxykinase (PEPCK)-in both

RV and left ventricle (LV) from five controls and five ARVC patients. In vitro motility assays were used to analyse functional properties of myosin. In the RV, sliding velocity was nearly two-fold lower in ARVC than in controls, whereas a 10% reduction in velocity values was noted between ARVC and non-failing myocardium in the LV. In controls, PPARalpha and MCAD mRNA and protein levels were higher in the RV compared with the LV. In ARVC, the expression of PPARalpha and MCAD mRNA and/or proteins was decreased in both RV and LV. RV from ARVC was also characterized by a dramatic activation of the PPARgamma pathway, as attested by the increase in PPARgamma mRNA and protein (500 and 270%, respectively, each $P < 0.001$) and by the induction of PEPCK gene. In contrast, the LV of ARVC heart exhibited no changes in the expression of the PPARgamma regulatory pathway compared with control. CONCLUSION: ARVC is associated with major disturbances in the PPARalpha and PPARgamma signalling pathway in the RV that may contribute to intracellular lipid overload and severe myosin dysfunction.

PECQUEUR C., ALVES-GUERRA C., RICQUIER D., BOUILLAUD F.

UCP2, a metabolic sensor coupling glucose oxidation to mitochondrial metabolism ?

IUMB Life, 61 (7), 762-767, 2009

(Services cités : [FRE 3210](#))

Mitochondrial uncoupling of oxidative phosphorylation may serve a variety of purposes such as the regulation of substrate oxidation, free radical production (a major by-product of mitochondrial respiration) and ATP production and turnover. As regulators of energy expenditure and antioxidant defenses, uncoupling proteins would seem to offer an attractive mechanism by which to explain the control of body weight, resting metabolic rate and aging. As a result, the discovery of UCP1 homologues has led to an impressive number of publications. However, 10 years after their identification, no consensus has been found concerning the function of UCP homologues, and there are controversies as to whether or not they even have physiologically significant uncoupling activity. Here, we discuss a potential new function for UCP2, as a carrier involved in the coupling between glucose oxidation and mitochondrial metabolism. (c) 2009 IUBMB IUBMB Life 61(7): 762-767, 2009.

RIGOURD V., CHELBI S., CHAUVET C., REBOURCET R., BARBAUX S., BESSIERES B., MONDON F., MIGNOT T.M., DANAN J.L., VAIMAN D.

Re-evaluation of the role of STOX1 transcription factor in placental development and preeclampsia.

J. Reprod. Immunol., 82 (2), 174-181, 2009

(Services cités : [FRE 3210](#))

Preeclampsia is a common disease of pregnancy, characterized by high blood pressure and proteinuria appearing from the second trimester of gestation. Preeclampsia has been shown to have a strong genetic component. In 2005 a positional cloning project led to the discovery of the STOX1 transcription factor, and mutations of this gene were proposed as causal for preeclampsia in Dutch families. Despite the publication of three contradictory studies, we have shown by analyzing the functional effects of STOX1 that its overexpression in choriocarcinoma cells recapitulates several transcriptomic aspects of preeclampsia. In this review, the current literature is analyzed to evaluate the possible involvement of STOX1 in the pathogenesis of this disease. While preeclampsia obviously cannot be considered as a disease caused by mutation in a single gene, we argue that STOX1 may be at the center of common pathways leading to preeclampsia.

2008

BASTIN J., AUBEY F., ROTIG A., MUNNICH A., DJOUADI F.

Activation of peroxisome proliferator activated receptor pathway stimulates the mitochondrial respiratory chain and can correct deficiencies in patients' cells lacking its components.

J. Clin. Endocrinol. Metabol., 93 (4), 1433-1441, 2008

(Services cités : U781, UPR 9078)

Context: The mitochondrial Respiratory Chain (RC) disorders are the largest group of inborn errors of metabolism and still remain without treatment in most cases. Objective: We tested whether bezafibrate, a drug acting as a Peroxisome Proliferator Activated Receptor (PPAR) agonist, could stimulate RC capacities. Design: Fibroblasts or myoblasts from controls or patients deficient in complex I (CI), complex III (CIII), or complex IV (CIV) were cultured with or without bezafibrate. Main outcomes measures: Enzyme activities, mRNA and protein expression and respiration rates were measured. Results: In control cells, bezafibrate increased the CI, CIII and CIV enzyme activities (+42 to +52%), as well as RC mRNAs (+40 to +120%) and RC protein levels (+50 to +150%). 9 out of 14 patient cells lines tested exhibited a significant increase in the activity of the deficient RC complex after bezafibrate treatment (+46 to +133%) and full pharmacological correction could be achieved in 7 cell lines. Similar effects were obtained using a PPARdelta agonist. These changes were related to a drug-induced increase in the mutated mRNAs and RC protein levels. Finally, the molecular mechanisms by which the PPAR pathway could induce the expression of genes encoding structural subunits or ancillary proteins of the RC apparatus, leading to stimulate the activity and protein levels of RC complex, likely involved the co-activator PGC-1alpha. Conclusions: This study suggests a rationale for a possible correction of moderate RC disorders due to mutations in nuclear genes, using existing drugs, and brings new insights into the role of PPAR in the regulation of mitochondrial RC in human cells.

DJOUADI F., BASTIN J.

PPARs as therapeutic targets for correction of inborn mitochondrial fatty acid oxidation disorders.

J. Inherit. Metab. Dis., 31 (2), 217-225, 2008

(Services cités : UPR 9078)

Enzyme defects in the mitochondrial fatty acid oxidation (FAO) are a large family of inherited metabolic disease well characterized clinically and genetically, but for which pharmacological strategies remain limited. It is now well established that regulation of genes involved in mitochondrial FAO is under control of the PPAR (peroxisome proliferator activated receptor) signalling pathway, and this led us to test a possible pharmacological correction of FAO disorders by fibrates and other PPAR activators. This review presents the basic data supporting our initial hypothesis, summarizes the results obtained in cells from patients with CPT II (carnitine palmitoyltransferase II) or VLCAD (very long-chain acyl-CoA dehydrogenase) deficiency, and discusses the perspectives and limits of this approach for therapy of these disorders.

GONZALEZ-BARROSO-MADRID M.D.M., GIURGEA I., BOUILLAUD F., ANEDDA A., BELLANNE-CHANTELOT C., HUBERT L., de KEYZER Y., de LONLAY P.,

RICQUIER D.

Mutations in UCP2 in congenital hyperinsulinism reveal a role for regulation of insulin secretion. *PLoS ONE*, 3 (12), e3850, 2008

(Services cités : U781, UPR 9078, Département de Pédiatrie)

Although the most common mechanism underlying congenital hyperinsulinism is dysfunction of the pancreatic ATP-sensitive potassium channel, the pathogenesis and genetic origins of this disease remains largely unexplained in more than half of all patients. UCP2 knockout mice exhibit an hyperinsulinemic hypoglycemia, suggesting an involvement of UCP2 in insulin secretion. However, a possible pathogenic role for UCP2 protein in the development of human congenital hyperinsulinism or of any human disease has not yet been investigated. We studied ten children exhibiting congenital hyperinsulinism, without detectable mutations in the known congenital hyperinsulinism-causing genes. Parental-inherited heterozygous UCP2 variants encoding amino-acid changes were found in two unrelated children with congenital hyperinsulinism. Functional assays in yeast and in insulin-secreting cells revealed an impaired activity of UCP2 mutants. Therefore, we report the finding of UCP2 coding variants in human congenital hyperinsulinism, which reveals a role for this gene in the regulation of insulin secretion and glucose metabolism in humans. Our results show for the first time a direct association between UCP2 amino acid alteration and human disease and highlight a role for mitochondria in hormone secretion.

NUBEL T., EMRE Y., RABIER D., CHADEFAX B., RICQUIER D., BOUILLAUD F.

Modified glutamine catabolism in macrophages of Ucp2 knock-out mice.

Biochim. Biophys. Acta - Bioenergetics, 1777 (1), 48-54, 2008

(Services cités : Biochimie Métabolique, UPR 9078)

Uncoupling protein 2 (UCP2) belongs to a family of transporters of the mitochondrial inner membrane and is reported to uncouple respiration from ATP synthesis. Our observation that the amino acid glutamine specifically induces UCP2 protein expression prompted us to investigate metabolic consequences of a UCP2 knockdown (Ucp2-KO) when glutamine is offered as a substrate. We found that Ucp2-KO macrophages incubated in the presence of glutamine exhibit a lower ammonium release, a decreased respiratory rate, and an intracellular accumulation of aspartate. Therefore, we conclude that UCP2 expression is required for efficient oxidation of glutamine in macrophages. This role of UCP2 in glutamine metabolism appears independent from the uncoupling activity of UCP2.

PECQUEUR C., BUI T., GELLY C., HAUCHARD J., BARBOT C., BOUILLAUD F., RICQUIER D., MIROUX B., THOMPSON C.B.

Uncoupling protein-2 controls proliferation by promoting fatty acid oxidation and limiting glycolysis-derived pyruvate utilization.

FASEB J., 22 (1), 9-18, 2008

(Services cités : UPR 9078)

Uncoupling protein-2 (UCP2) belongs to the mitochondrial carrier family and has been thought to be involved in suppressing mitochondrial ROS production through uncoupling mitochondrial respiration from ATP synthesis. However, we show here that loss of function of UCP2 does not result in a significant increase in ROS production or an increased propensity for cells to undergo senescence in culture. Instead, Ucp2^{-/-} cells display enhanced proliferation associated with a

metabolic switch from fatty acid oxidation to glucose metabolism. This metabolic switch requires the unrestricted availability of glucose, and Ucp2^{-/-} cells more readily activate autophagy than wild-type cells when deprived of glucose. Altogether, these results suggest that UCP2 promotes mitochondrial fatty acid oxidation while limiting mitochondrial catabolism of pyruvate. The persistence of fatty acid catabolism in Ucp2^{+/+} cells during a proliferative response correlates with reduced cell proliferation and enhances resistance to glucose starvation-induced autophagy.- Pecqueur, C., Bui, T., Gelly, C., Hauchard, J., Barbot, C., Bouillaud, F., Ricquier, D., Miroux, B., Thompson, C. B. Uncoupling protein-2 controls proliferation by promoting fatty acid oxidation and limiting glycolysis-derived pyruvate utilization.

SILVAIN M., BLIGNY D., APARICIO T., LAFORET P., GRODET A., PERETTI N., MENARD D., DJOUADI F., JARDEL C., BEGUE J.M., WALKER F., SCHMITZ J., LACHAUX A., AGGERBECK L.P., SAMSON-BOUMA M.E.

Anderson's disease (chylomicron retention disease): a new mutation in the SARA2 gene associated with muscular and cardiac abnormalities.

Clin. Genet., 74 (6), 546-552, 2008

(Services cités : Département de Pédiatrie, U781, UPR 9078)

Anderson's disease (AD) or chylomicron retention disease (CMRD) is a rare hereditary lipid malabsorption syndrome linked to SARA2 gene mutations. We report in this study a novel mutation in two sisters for which the Sar1b protein is predicted to be truncated by 32 amino acids at its carboxyl-terminus. Because the SARA2 gene is also expressed in the muscle, heart, liver and placenta, extraintestinal clinical manifestations may exist. For the first time, we describe in this study in the two sisters muscular as well as cardiac abnormalities that could be related to the reported expression of SARA2 in these tissues. We also evaluated six other patients for potential manifestations of the SARA2 mutation. The creatine phosphokinase levels were increased in all patients [1.5-9.4 x normal (N)] and transaminases were moderately elevated in five of the eight patients (1.2-2.6 x N), probably related to muscle disease rather than to liver dysfunction. A decreased ejection fraction occurred in one patient (40%, N: 60%). The muscle, liver and placental tissues that were examined had no specific abnormalities and, in particular, no lipid accumulation. These results suggest that myolysis and other extraintestinal abnormalities can occur in AD/CMRD and that the clinical evaluation of patients should reflect this.

ZOONENS M.A., RESHETNYAK Y.K., ENGELMAN D.M.

Bilayer interactions of pHLIP, a peptide that can deliver drugs and target tumors.

Biophys. J., 95 (1), 225-235, 2008

(Services cités : UPR 9078)

The pH-dependent insertion of pHLIP (pH Low Insertion Peptide) across membranes is proving to be a useful property for targeting acidic tissues or tumors and delivering drugs attached to its C-terminus. It also serves as a model peptide for studies of protein insertion into membranes, so further elucidation of the insertion mechanism of pHLIP and its features is desirable. Here, we examine how the peptide perturbs a model phosphatidylcholine membrane and how it associates with the lipid bilayer using an array of fluorescence techniques, including fluorescence anisotropy measurements of TMA-DPH anchored in bilayers, quenching of pHLIP fluorescence by brominated lipids and acrylamide, and measurements of energy transfer between aromatic residues of pHLIP and TMA-DPH. When pHLIP is bound to the surface of bilayers near neutral

pH, the membrane integrity is preserved while the elastic properties of bilayers are changed as reported by an increase of membrane viscosity. When it is inserted, there is little perturbation of the lipids. The results also suggested that pHLIP can bind to the membrane surface in a shallow or a deep mode depending on the phase state of lipids. Using the parallax analysis, the change of the penetration depth of pHLIP was estimated to be 0.4 Å from the bilayer center and 2.8 Å from the membrane surface after the liquid-to-gel phase transition.

2007

ARSENIJEVIC D., CLAVEL S., SANCHIS D., PLAMONDON J., HUANG Q., RICQUIER D., ROUGER L., RICHARD D.

Induction of Ucp2 expression in brain phagocytes and neurons following murine toxoplasmosis: an essential role of IFN-gamma and an association with negative energy balance.

J. Neuroimmunol., 186 (1-2), 121-132, 2007

(Services cités : UPR 9078)

A model of murine toxoplasmosis was used to study cellular and temporal expression of uncoupling protein-2 (Ucp2) in the brain. In situ hybridization indicated that Ucp2 was located in neurons. Nuclei structures involved in energy balance, in particular the nucleus of the solitary tract (NST), was shown to have a positive association between negative energy balance and Ucp2 levels. Infection-induced Ucp2 expression colocalized mainly with microglial cells, but also with infiltrating macrophages and neutrophils in the brain, which was evident from day 9 post-infection. Using cytokine knockout mice we demonstrate that microglial Ucp2 induction in the brain was largely dependant on interferon-gamma, but not interleukin-6 or tumour-necrosis-factor-alpha in response to infection. In summary, this study shows that Ucp2 is regulated in a different manner in neurons than in microglia/phagocytes following infection. Our study indicates that an association exists between negative energy balance and neuronal Ucp2 levels in the NST, in particular.

BENSALEM N., MASSCHELEYN S., MOZO J., VALLEE B., BROUILLARD F., TRUDEL S., RICQUIER D., EDELMAN A., GUERRERA I.C., MIROUX B.

High Sensitivity Identification of Membrane Proteins by MALDI TOF-MASS Spectrometry Using Polystyrene Beads.

J. Proteome Res., 6 (4), 1595-1602, 2007

(Services cités : IRNEM, U845 (AE), UPR 9078)

Membrane proteins play a large variety of functions in life and represent 30% of all genomes sequenced. Due to their hydrophobic nature, they are tightly bound to their biological membrane, and detergents are always required to extract and isolate them before identification by mass spectrometry (MS). The latter, however remains difficult. Peptide mass fingerprinting methods using techniques such as MALDI-TOF MS, for example, have become an important analytical tool in the identification of proteins. However, PMF of membrane proteins is a real challenge for at least three reasons. First, membrane proteins are naturally present at low levels; second, most of the detergents strongly inhibit proteases and have deleterious effects on MALDI spectra; and third, despite the presence of detergent, membrane proteins are unstable and often aggregate. We took the mitochondrial uncoupling protein 1 (UCP1) as a model and showed that differential acetonitrile extraction of tryptic peptides combined with the use of polystyrene Bio-Beads triggered high resolution of the MALDI-TOF identification of mitochondrial membrane proteins

solubilized either with Triton-X100 or CHAPS detergents. Keywords: mass spectrometry * detergent * membrane protein identification * peptide mass fingerprinting.

BOURGEON S., RACLOT T., LE MAHO Y., RICQUIER D., CRISCUOLO F.

Innate immunity, assessed by plasma NO measurements, is not suppressed during the incubation fast in eiders.

Dev. Comparative Immunol., 31 (7), 720-728, 2007

(Services cités : [UPR 9078](#))

Immunity is hypothesized to share limited resources with other physiological functions and may mediate life history trade-offs, for example between reproduction and survival. However, vertebrate immune defense is a complex system that consists of three components. To date, no study has assessed all of these components for the same animal model and within a given situation. Previous studies have determined that the acquired immunity of common eiders (*Somateria mollissima*) is suppressed during incubation. The present paper aims to assess the innate immune response in fasting eiders in relation to their initial body condition. Innate immunity was assessed by measuring plasma nitric oxide (NO) levels, prior to and after injection of lipopolysaccharides (LPS), a method which is easily applicable to many wild animals. Body condition index and corticosterone levels were subsequently determined as indicators of body condition and stress level prior to LPS injection. The innate immune response in eiders did not vary significantly throughout the incubation period. The innate immune response of eiders did not vary significantly in relation to their initial body condition but decreased significantly when corticosterone levels increased. However, NO levels after LPS injection were significantly and positively related to initial body condition, while there was a significant negative relationship with plasma corticosterone levels. Our study suggests that female eiders preserve an effective innate immune response during incubation and this response might be partially determined by the initial body condition.

CHEVILLOTTE E., GIRALT M., MIROUX B., RICQUIER D., VILLARROYA F.

Uncoupling protein-2 controls adiponectin gene expression in adipose tissue through the modulation of reactive oxygen species production.

Diabetes, 56 (4), 1042-1050, 2007

(Services cités : [UPR 9078](#))

Uncoupling protein-2 (UCP2) is a mitochondrial membrane transporter expressed in white adipose tissue. We observed that circulating adiponectin levels and adiponectin gene expression in adipose tissue are reduced in UCP2-null mice. We studied whether mitochondrial activity and its control by UCP2 may regulate adiponectin gene expression. In 3T3-L1 cells, increasing UCP2 mitochondrial levels by adenoviral-mediated gene transfer induced adiponectin gene expression, whereas oligomycin and antimycin A, inhibitors of ATP synthesis and mitochondrial respiration, led to a downregulation. Reactive oxygen species (ROS) scavengers alleviated the repression of adiponectin gene expression caused by oligomycin or antimycin A. The action of ROS involves the transcription factor CHOP-10, the abundance of which was reduced in response to UCP2 and was induced by oligomycin. CHOP-10 inhibited adiponectin gene expression by interfering with the -117/-73 CCAAT/enhancer binding protein-binding region in the adiponectin gene promoter. Moreover, CHOP-10 levels were increased in adipose tissue from UCP2-null mice. Results indicate that the modulation of ROS levels by mitochondrial activity, and specifically as a

consequence of the action of UCP2, controls adiponectin gene expression. This provides a physiological mechanism by which the adipose tissue energetic status may determine the extent of adiponectin release and influence systemic insulin sensitivity.

COPPOLA A., LIU Z.W., ANDREWS Z.B., PARADIS E., ROY M.C., FRIEDMAN J.M., RICQUIER D., RICHARD D., HORVATH T.L., GAO X.B., DIANO S.

A Central Thermogenic-like Mechanism in Feeding Regulation: An Interplay between Arcuate Nucleus T3 and UCP2.

Cell Metab., 5 (1), 21-33, 2007

(Services cités : [UPR 9078](#))

The active thyroid hormone, triiodothyronine (T3), regulates mitochondrial uncoupling protein activity and related thermogenesis in peripheral tissues. Type 2 deiodinase (DII), an enzyme that catalyzes active thyroid hormone production, and mitochondrial uncoupling protein 2 (UCP2) are also present in the hypothalamic arcuate nucleus, where their interaction and physiological significance have not been explored. Here, we report that DII-producing glial cells are in direct apposition to neurons coexpressing neuropeptide Y (NPY), agouti-related protein (AgRP), and UCP2. Fasting increased DII activity and local thyroid hormone production in the arcuate nucleus in parallel with increased GDP-regulated UCP2-dependent mitochondrial uncoupling. Fasting-induced T3-mediated UCP2 activation resulted in mitochondrial proliferation in NPY/AgRP neurons, an event that was critical for increased excitability of these orexigenic neurons and consequent rebound feeding following food deprivation. These results reveal a physiological role for a thyroid-hormone-regulated mitochondrial uncoupling in hypothalamic neuronal networks.

DAGONEAU N., BELLAIS S., BLANCHET P., SARDA P., AL-GAZALI L.I., DI ROCCO M., HUBER C., DJOUADI F., LE GOFF C., MUNNICH A., CORMIER-DAIRE V.

Mutations in Cytokine Receptor-Like Factor 1 (CRLF1) Account for Both Crisponi and Cold-Induced Sweating Syndromes.

Amer. J. Hum. Genet., 80 (5), 966-970, 2007

(Services cités : [Génétique Médicale Pédiatrique](#), [UPR 9078](#))

Crisponi syndrome is a rare autosomal recessive disorder characterized by congenital muscular contractions of facial muscles, with trismus in response to stimuli, dysmorphic features, bilateral camptodactyly, major feeding and respiratory difficulties, and access of hyperthermia leading to death in the first months of life. The overlap with Stuve-Wiedemann syndrome (SWS) is striking, but the two conditions differ in that congenital lower limb bowing is absent in Crisponi syndrome, whereas it is a cardinal feature of SWS. We report here the exclusion of the leukemia inhibitory factor receptor gene in Crisponi syndrome and the identification of homozygote or compound heterozygote cytokine receptor-like factor 1 (CRLF1) mutations in four children from three unrelated families. The four mutations were located in the immunoglobulin-like and type III fibronectin domains, and three of them predicted premature termination of translation. Using real-time quantitative polymerase chain reaction, we found a significant decrease in CRLF1 mRNA expression in patient fibroblasts, which is suggestive of a mutation-mediated decay of the abnormal transcript. CRLF1 forms a heterodimer complex with cardiotrophin-like cytokine factor 1, and this heterodimer competes with ciliary neurotrophic factor for binding to the ciliary neurotrophic factor receptor (CNTFR) complex. The identification of CRLF1 mutations in Crisponi syndrome supports the key role of the CNTFR pathway in the function of the autonomic

nervous system.

DANSAULT A., DAVID G., SCHWARTZ C., JALIFFA C., VIEIRA V., de LA HOUSSAYE G., BIGOT K., CATIN F., TATTU L., CHOPIN C., HALIMI P., ROCHE O., VAN REGEMORTER N., MUNIER F., SCHORDERET D., DUFIER J.L., MARSAC C., RICQUIER D., MENASCHE M., PENFORNIS A., ABITBOL M.

Three new PAX6 mutations including one causing an unusual ophthalmic phenotype associated with neurodevelopmental abnormalities.

Mol. Vis., 13 (.), 511-523, 2007

(Services cités : CERTO, UPR 9078, Ophthalmologie)

PURPOSE: The PAX6 gene was first described as a candidate for human aniridia. However, PAX6 expression is not restricted to the eye and it appears to be crucial for brain development. We studied PAX6 mutations in a large spectrum of patients who presented with aniridia phenotypes, Peters' anomaly, and anterior segment malformations associated or not with neurological anomalies. **METHODS:** Patients and related families were ophthalmologically phenotyped, and in some cases neurologically and endocrinologically examined. We screened the PAX6 gene by direct sequencing in three groups of patients: those affected by aniridia; those with diverse ocular manifestations; and those with Peters' anomaly. Two mutations were investigated by generating crystallographic representations of the amino acid changes. **RESULTS:** Three novel heterozygous mutations affecting three unrelated families were identified: the g.572T>C nucleotide change, located in exon 5, and corresponding to the Leucine 46 Proline amino-acid mutation (L46P); the g.655A>G nucleotide change, located in exon 6, and corresponding to the Serine 74 Glycine amino-acid mutation (S74G); and the nucleotide deletion 579delG del, located in exon 6, which induces a frameshift mutation leading to a stop codon (V48fsX53). The L46P mutation was identified in affected patients presenting bilateral microphthalmia, cataracts, and nystagmus. The S74G mutation was found in a large family that had congenital ocular abnormalities, diverse neurological manifestations, and variable cognitive impairments. The 579delG deletion (V48fsX53) caused in the affected members of the same family bilateral aniridia associated with congenital cataract, foveal hypoplasia, and nystagmus. We also detected a novel intronic nucleotide change, IVS2+9G>A (very likely a mutation) in an apparently isolated patient affected by a complex ocular phenotype, characterized primarily by a bilateral microphthalmia. Whether this nucleotide change is indeed pathogenic remains to be demonstrated. Two previously known heterozygous mutations of the PAX6 gene sequence were also detected in patients affected by aniridia: a de novo previously known nucleotide change, g.972C>T (Q179X), in exon 8, leading to a stop codon and a heterozygous g.555C>A (C40X) recurrent nonsense mutation in exon 5. No mutations were found in patients with Peters' anomaly. **CONCLUSIONS:** We identified three mutations associated with aniridia phenotypes (Q179X, C40X, and V48fsX53). The three other mutations reported here cause non-aniridia ocular phenotypes associated in some cases with neurological anomalies. The IVS2+9G>A nucleotide change was detected in a patient with a microphthalmia phenotype. The L46P mutation was detected in a family with microphthalmia, cataract, and nystagmus. This mutation is located in the DNA-binding paired-domain and the crystallographic representations of this mutation show that this mutation may affect the helix-turn-helix motif, and as a consequence the DNA-binding properties of the resulting mutated protein. Ser74 is located in the PAX6 PD linker region, essential for DNA recognition and DNA binding, and the side chain of the Ser74 contributes to DNA recognition by the linker domain through direct contacts. Crystallographic

representations show that the S74G mutation results in no side chain and therefore perturbs the DNA-binding properties of PAX6. This study highlights the severity and diversity of the consequences of PAX6 mutations that appeared to result from the complexity of the PAX6 gene structure, and the numerous possibilities for DNA binding. This study emphasizes the fact that neurodevelopmental abnormalities may be caused by PAX6 mutations. The neuro-developmental abnormalities caused by PAX6 mutations are probably still overlooked in the current clinical examinations performed throughout the world in patients affected by PAX6 mutations.

DUVILLIE B., HEINIS M., STETSYUK V.

In vivo and in vitro Techniques to Study Pancreas Development and Islet Cell Function.

Endocr. Dev., 12 (.), 46-54, 2007

(Services cités : UPR 9078)

Over the last decades, pancreas development has been widely investigated. Understanding the mechanisms that control Beta-cell development should allow progress towards the regeneration of these cells in humans. Particularly, it is well established that inductive signals from the mesenchyme play an essential role in the proliferation of precursor cells. In the present review, we focused on the roles of fibroblast growth factors (FGFs) in pancreas development. Improvements of the in vivo and in vitro techniques were used to define the function of FGF10. Experiments on FGF10 knockout mice showed that FGF10 is required for the proliferation of precursor cells and the pancreas development. Several laboratories used different in vitro techniques to study the effect of FGF10 on Beta-cell differentiation. These methods of investigation are described here. In our experiments, pancreases were placed at the air-liquid interface to define the precise mechanism of action of FGF10. We showed that FGF10 positively regulates the Beta-cell mass by increasing the proliferation of the early precursors and by extending the window of expression of the endocrine precursor marker Ngn3. These data are compared with studies performed with other culture systems. Finally, the role of other FGFs is discussed.

EMRE Y., HURTAUD C., NUBEL T., CRISCUOLO F., RICQUIER D., CASSARD-DOULCIER A.M.

Mitochondria contribute to LPS-induced MAPK activation via uncoupling protein UCP2 in macrophages.

Biochem. J., 402 (2), 271-278, 2007

(Services cités : UPR 9078)

The mitochondrion is a major organelle contributing to energy metabolism but also a main site of ROS (reactive oxygen species) production. LPS (lipopolysaccharide)-induced ROS signalling is a critical event in macrophage activation. In the present paper we report that part of LPS-mediated ROS signalling comes from mitochondria inside a signal amplification loop that enhances MAPK (mitogen-activated protein kinase) activation. More precisely, we have identified the inner mitochondrial membrane UCP2 (uncoupling protein 2) as a physiological brake on ROS signalling. Stimulation of murine bone marrow-derived macrophages by LPS quickly down-regulated UCP2 through the JNK (c-Jun N-terminal kinase) and p38 pathways. UCP2 down-regulation was shown to be necessary to increase mitochondrial ROS production in order to potentiate MAPK activation. Consistent with this, UCP2-deficient macrophages exhibit an enhanced inflammatory state characterized by increased nitric oxide production and elevated

migration ability. Additionally, we found that the absence of UCP2 renders macrophages more resistant to nitric oxide-induced apoptosis.

EMRE Y., HURTAUD C., KARACA M., NUBEL T., ZAVALA F., RICQUIER D.

Role of uncoupling protein UCP2 in cell-mediated immunity: How macrophage-mediated insulinitis is accelerated in a model of autoimmune diabetes.

Proc. Nat. Acad. Sci. USA, *104* (48), 19085-19090, 2007

(Services cités : [UMR 8147](#), [UPR 9078](#))

Infiltration of inflammatory cells into pancreatic islets of Langerhans and selective destruction of insulin-secreting beta-cells are characteristics of type 1 diabetes. Uncoupling protein 2 (UCP2) is a mitochondrial protein expressed in immune cells. UCP2 controls macrophage activation by modulating the production of mitochondrial reactive oxygen species (ROS) and MAPK signaling. We investigated the role of UCP2 on immune cell activity in type 1 diabetes in Ucp2-deficient mice. Using the model of multiple low-dose streptozotocin (STZ)-induced diabetes, we found that autoimmune diabetes was strongly accelerated in Ucp2-KO mice, compared with Ucp2-WT mice with increased intraislet lymphocytic infiltration. Macrophages from STZ-treated Ucp2-KO mice had increased IL-1beta and nitric oxide (NO) production, compared with WT macrophages. Moreover, more macrophages were recruited in islets of STZ-treated Ucp2-KO mice, compared with Ucp2-WT mice. This finding also was accompanied by increased NO/ROS-induced damage. Altogether, our data show that inflammation is stronger in Ucp2-KO mice and islets, leading to the exacerbated disease in these mice. Our results highlight the mitochondrial protein UCP2 as a new player in autoimmune diabetes.

EMRE Y., HURTAUD C., RICQUIER D., BOULLAUD F., HUGHES J., CRISCUOLO F.

Avian UCP: The Killjoy in the Evolution of the Mitochondrial Uncoupling Proteins.

J. Mol. Evol., *65* (4), 392-402, 2007

(Services cités : [UPR 9078](#))

The understanding of mitochondrial functioning is of prime importance since it combines the production of energy as adenosine triphosphate (ATP) with an efficient chain of redox reactions, but also with the unavoidable production of reactive oxygen species (ROS) involved in aging. Mitochondrial respiration may be uncoupled from ATP synthesis by a proton leak induced by the thermogenic uncoupling protein 1 (UCP1). Mild uncoupling activity, as proposed for UCP2, UCP3, and avian UCP could theoretically control ROS production, but the nature of their transport activities is far from being definitively understood. The recent discovery of a UCP1 gene in fish has balanced the evolutionary view of uncoupling protein history. The thermogenic proton transport of mammalian UCP1 seems now to be a late evolutionary characteristic and the hypothesis that ancestral UCPs may carry other substrates is tempting. Using in silico genome analyses among taxa and a biochemical approach, we present a detailed phylogenetic analysis of UCPs and investigate whether avian UCP is a good candidate for pleiotropic mitochondrial activities, knowing that only one UCP has been characterized in the avian genome, unlike all other vertebrates. We show, here, that the avian class seems to be the only vertebrate lineage lacking two of the UCP1/2/3 homologues present in fish and mammals. We suggest, based on phylogenetic evidence and synteny of the UCP genes, that birds have lost UCP1 and UCP2. The phylogeny also supports the history of two rounds of duplication during vertebrate evolution. The avian uncoupling protein then represents a unique opportunity to explore how UCPs' activities

are controlled, but also to understand why birds exhibit such a particular relationship between high metabolism and slow rate of aging.

FRONTINI A., ROUSSET S., CASSARD-DOULCIER A.M., ZINGARETTI C., RICQUIER D., CINTI S.

Thymus uncoupling protein 1 is exclusive to typical brown adipocytes and is not found in thymocytes.

J. Histochem. Cytochem., 55 (2), 183-189, 2007

(Services cités : [UPR 9078](#))

A large number of studies have established the mitochondrial uncoupling protein UCP1 as a specific marker of brown adipocytes, where it controls energy dissipation of fatty acid oxidation as heat in response to physiological requirements. Following the recent report of the detection of UCP1 in thymocytes of rats and mice, we reinvestigated its presence in thymus. Light microscopy and immunohistochemical analysis demonstrated that the UCP1 signal in thymus is entirely explained by the presence of typical brown adipocytes around the gland. Staining for UCP1 was not observed in thymocytes. Similarly, UCP1 failed to be observed in rat spleen, skeletal muscle, stomach, intestine, or uterus, even after exposure of animals to the cold. These data confirm the specificity of UCP1 expression in the thermogenic brown adipocytes and argue against a direct role for this mitochondrial transporter in immune cells. Whether brown adipocytes adjacent to thymic lobes play a role in thymus physiology remains to be investigated.

GAMBERT S., RICQUIER D.

Mitochondrial thermogenesis and obesity.

Curr. Opin. Clin. Nutr. Metab. Care, 10 (6), 664-670, 2007

(Services cités : [UPR 9078](#))

The clinical and research implications of these findings are that the mechanisms inhibiting adaptive thermogenesis during diet restriction should be investigated. An important field of research is the contribution of transcriptional coactivators to adipocyte plasticity since adipocytes have an underestimated ability to oxidise fatty acids in addition to their role in triglyceride storage.

GOBIN-LIMBALLE S., DJOUADI F., AUBEY F., OLPIN S., ANDRESEN B.S., YAMAGUCHI S., MANDEL H., FUKAO T., RUITER J.P., WANDERS R.J., MCANDREW R., KIM J.J., BASTIN J.

Genetic basis for correction of very-long-chain acyl-coenzyme A dehydrogenase deficiency by bezafibrate in patient fibroblasts: toward a genotype-based therapy.

Amer. J. Hum. Genet., 81 (6), 1133-1143, 2007

(Services cités : [UPR 9078](#))

Very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) deficiency is an inborn mitochondrial fatty-acid beta -oxidation (FAO) defect associated with a broad mutational spectrum, with phenotypes ranging from fatal cardiopathy in infancy to adolescent-onset myopathy, and for which there is no established treatment. Recent data suggest that bezafibrate could improve the FAO capacities in beta -oxidation-deficient cells, by enhancing the residual level of mutant enzyme activity via gene-expression stimulation. Since VLCAD-deficient

patients frequently harbor missense mutations with unpredictable effects on enzyme activity, we investigated the response to bezafibrate as a function of genotype in 33 VLCAD-deficient fibroblasts representing 45 different mutations. Treatment with bezafibrate (400 μ M for 48 h) resulted in a marked increase in FAO capacities, often leading to restoration of normal values, for 21 genotypes that mainly corresponded to patients with the myopathic phenotype. In contrast, bezafibrate induced no changes in FAO for 11 genotypes corresponding to severe neonatal or infantile phenotypes. This pattern of response was not due to differential inductions of VLCAD messenger RNA, as shown by quantitative real-time polymerase chain reaction, but reflected variable increases in measured VLCAD residual enzyme activity in response to bezafibrate. Genotype cross-analysis allowed the identification of alleles carrying missense mutations, which could account for these different pharmacological profiles and, on this basis, led to the characterization of 9 mild and 11 severe missense mutations. Altogether, the responses to bezafibrate reflected the severity of the metabolic blockage in various genotypes, which appeared to be correlated with the phenotype, thus providing a new approach for analysis of genetic heterogeneity. Finally, this study emphasizes the potential of bezafibrate, a widely prescribed hypolipidemic drug, for the correction of VLCAD deficiency and exemplifies the integration of molecular information in a therapeutic strategy.

GOUBERN M., ANDRIAMIHAJA M., NUBEL T., BLACHIER F., BOUILLAUD F.

Sulfide, the first inorganic substrate for human cells.

FASEB J., 21 (8), 1699-1706, 2007

(Services cités : UPR 9078)

Hydrogen sulfide (H₂S) is produced inside the intestine and is known as a poison that inhibits cellular respiration at the level of cytochrome oxidase. However, sulfide is used as an energetic substrate by many photo- and chemoautotrophic bacteria and by animals such as the lugworm *Arenicola marina*. The concentrations of sulfide present in their habitats are comparable with those present in the human colon. Using permeabilized colonic cells to which sulfide was added by an infusion pump we show that the maximal respiratory rate of colonocyte mitochondria in presence of sulfide compares with that obtained with succinate or L-alpha-glycerophosphate. This oxidation is accompanied by mitochondrial energization. In contrast, other cell types not naturally exposed to high concentration of sulfide showed much lower oxidation rates.

Mitochondria showed a very high affinity for sulfide that permits its use as an energetic substrate at low micromolar concentrations, hence, below the toxic level. However, if the supply of sulfide exceeds the oxidation rate, poisoning renders mitochondria inefficient and our data suggest that an anaerobic mechanism involving partial reversion of Krebs cycle already known in invertebrates takes place. In conclusion, this work provides additional and compelling evidence that sulfide is not only a toxic compound. According to our study, sulfide appears to be the first inorganic substrate for mammalian cells characterized thus far.

HURTAUD C., GELLY C., CHEN Z., LEVI-MEYRUEIS C., BOUILLAUD F.

Glutamine stimulates translation of uncoupling protein 2mRNA.

Cell. Mol. Life Sci., 64 (14), 1853-1860, 2007

(Services cités : UPR 9078)

Uncoupling protein 2 (UCP2) belongs to a family of transporters/exchangers of the mitochondrial inner membrane. Using cell lines representing natural sites of UCP2 expression (macrophages,

colonocytes, pancreatic beta cells), we show that UCP2 expression is stimulated by glutamine at physiological concentrations. This control is exerted at the translational level. We demonstrate that the upstream open reading frame (ORF1) in the 5' untranslated region (5'UTR) of the UCP2 mRNA is required for this stimulation to take place. Cloning of the 5' UTR of the UCP2 mRNA in front of a GFP cDNA resulted in a reporter gene with which GFP expression could be induced by glutamine. An effect of glutamine on translation of a given mRNA has not been identified before, and this is the first evidence for a link between UCP2 and glutamine, an amino acid oxidized by immune cells or intestinal epithelium and playing a role in the control of insulin secretion.

ROUSSET S., MOZO J., DUJARDIN G., EMRE Y., MASSCHELEYN S., RICQUIER D., CASSARD-DOULCIER A.M.

UCP2 is a mitochondrial transporter with an unusual very short half-life.

FEBS Lett., 581 (3), 479-482, 2007

(Services cités : UPR 9078)

This study focused on the stability of UCP2 (uncoupling protein 2), a mitochondrial carrier located in the inner membrane of mitochondrion. UCP2 is very unstable, with a half-life close to 30min, compared to 30h for its homologue UCP1, a difference that may highlight different physiological functions. Heat production by UCP1 in brown adipocytes is generally a long and adaptive phenomenon, whereas control of mitochondrial ROS by UCP2 needs more subtle regulation. We show that a mutation in UCP2 shown to modify its activity, actually decreases its stability.

SEIFEDDINE R., DREIEM A., TOMKIEWICZ C., FULCHIGNONI-LATAUD M.C., BRITO I., DANAN J.L., FAVAUDON V., BAROUKI R., MASSAAD-MASSADE L.

Hypoxia and estrogen co-operate to regulate gene expression in T-47D human breast cancer cells.

J. Steroid Biochem. Mol. Biol., 104 (3-5), 169-179, 2007

(Services cités : UPR 9078)

Experimental and clinical studies have shown that both estrogen (E2) and hypoxia (H) are involved in tumor development and progression. A study was undertaken to determine whether these factors could interact to modulate gene expression using a microarray approach. We screened the transcript levels of over 8000 genes in the estrogen receptor (ER α) positive T-47D human breast cancer cell lines maintained at 21% O₂ or 1% O₂ with or without E2 co-treatment. Treatment by E2 or hypoxia alone altered the expression of 26 and 9 genes, respectively, whilst the expression of 31 genes was modulated by the H-E2 combination. The majority (21/31 genes) underwent a down-regulation. Microarray data was validated for 19 by quantitative real-time PCR and a good correlation noted ($r^2=0.8$). Five out of these 19 genes were assayed for protein expression by Western blot. A correlation was also found between mRNA and protein levels. Statistical analysis showed that the gene expression modulation by the combined H and E2 treatment was additive in most cases, but for RasGRP2 and transferrin (TF) an antagonistic interaction was noted. The results demonstrate that hypoxic conditions and estrogen exposure interact to modulate the expression of a limited number of genes involved in cell growth and differentiation, angiogenesis, protein transport, metabolism and apoptosis.

YAKUBU D.P., MOSTYN A., WILSON V., PEARCE S., ALVES-GUERRA M.C., PECQUEUR C., MIROUX B., BUDGE H., STEPHENSON T., SYMONDS M.E.

Different effects of maternal parity, cold exposure and nutrient restriction in late pregnancy on the abundance of mitochondrial proteins in the kidney, liver and lung of postnatal sheep.

Reproduction, 133 (6), 1241-1252, 2007

(Services cités : UPR 9078)

Adaptation to the extrauterine environment at birth relies upon the onset of postnatal function and increased metabolism in the lungs, liver and kidney, mediated partly by activation of mitochondrial proteins such as the voltage-dependent anion channel (VDAC), cytochrome c and, in the lung only, uncoupling protein (UCP)2. The magnitude of adaptation is dependent on the maternal metabolic and endocrine environment. We, therefore, examined the influence of maternal cold exposure (MCE) induced by winter shearing of pregnant sheep in conjunction with nutrient restriction (NR; 50% reduction in maternal food intake from 110 days gestation up to term). The effect of parity was also examined, as the offspring of nulliparous mothers are growth restricted compared with multiparous offspring. All sheep were twin bearing. One twin was sampled after birth and its sibling at 30 days. In the lung, both MCE and maternal nulliparity enhanced UCP2 abundance. However, whilst VDAC abundance was decreased in both the offspring of nulliparous mothers and by NR, it was transiently raised by MCE. Kidney VDAC abundance was reduced by MCE and nulliparity, adaptations only influenced by NR in multiparous mothers. Cytochrome c abundance was raised by MCE and by NR in multiparous controls and raised in offspring of nulliparous mothers. Liver VDAC and cytochrome c abundance were transiently reduced by MCE and persistently lower in offspring of nulliparous mothers. In conclusion, changes in the maternal metabolic environment have marked tissue-specific effects on mitochondrial protein abundance in the lungs, liver and kidney that may be important in enabling the newborn to effectively adapt to the extrauterine environment.

2006

BERTRAND S., CRISCUOLO F., FAIVRE B., SORCI G.

Immune activation increases susceptibility to oxidative tissue damage in Zebra Finches.

Funct. Ecol., 20 (6), 1022-1027, 2006

(Services cités : UPR 9078)

1. The innate immune response involves the production of highly reactive molecules (reactive oxygen and nitrogen species, ROS and RNS). These toxic compounds can effectively destroy invading pathogens but can also, non-specifically, target host cells. Tissue damage caused by ROS and RNS can be substantial if the inflammatory response is overactive, potentially inducing a so-called immunopathology.2. In this study, we induced an inflammatory response in Zebra Finches by the way of a LPS injection (lipopolysaccharide of the cell wall of *Escherichia coli*), using as a control a group of birds injected with saline (phosphate-buffered saline). Body mass was measured both before and 24 h after the procedure. We also took a blood sample 24 h after injection, to measure the resistance of red blood cells to a standardized free radical attack. The experiment was repeated twice with different individuals to ascertain the repeatability of the results.3. We found that birds injected with LPS lost significantly more body mass during the 24 h that followed, compared with control individuals. Similarly, LPS individuals were more susceptible to oxidative tissue damage, as their red blood cells had a weaker resistance to a free-radical attack than the red blood cells of control birds.4. This result shows that the inflammatory response with the associated production of cytotoxic compounds can produce side-effects that

may potentially result in increased damage to host tissues, therefore representing an immunopathology cost.

CRISCUOLO F., MOZO J., HURTAUD C., NUBEL T., BOUILLAUD F.

UCP2, UCP3, avUCP, what do they do when proton transport is not stimulated? Possible relevance to pyruvate and glutamine metabolism.

Biochim. Biophys. Acta - Bioenergetics, 1757 (9-10), 1284-1291, 2006

(Services cités : UPR 9078)

Uncoupling proteins (UCPs) are specialized members of the mitochondrial transporter family. They allow passive proton transport through the mitochondrial inner membrane. This activity leads to uncoupling of mitochondrial respiration and to energy waste, which is well documented with UCP1 in brown adipose tissue. The uncoupling activity of the new UCPs (discovered after 1997), such as UCP2 and UCP3 in mammals or avUCP in birds, is more difficult to characterize. However, extensive data support the idea that the new UCPs are involved in the control of reactive oxygen species (ROS) generation. This fits with the hypothesis that mild uncoupling caused by the UCPs prevents ROS production. Activators and inhibitors regulate the proton transport activity of the UCPs. In the absence of activators of proton transport, the UCP allows the permeation of other ions. We suggest that this activity has physiological significance and, for example, UCP3 expressed in glycolytic muscle fibres may be a passive pyruvate transporter ensuring equilibrium between glycolysis and oxidative phosphorylation. Induction of UCP2 expression by glutamine strengthens the proposal that new UCPs could act to determine the choice of mitochondrial substrate. This would obviously have an impact on mitochondrial bioenergetics and ROS production.

DUC-GOIRAN P., MIGNOT T.M., ROBERT B., MACHAVOINE F., MONDON F., HAGNERE A.M., VACHER-LAVENU M.C., DANAN J.L., VAIMAN D., BENASSAYAG C., FERRE F.

Expression and localization of alpha-fetoprotein mRNA and protein in human early villous trophoblasts.

Placenta, 27 (8), 812-821, 2006

(Services cités : UMR 8147, UPR 9078)

Alpha-fetoprotein (AFP) is a major plasma protein produced during human fetal life. It is a good marker for several possible disorders affecting gestation. We previously reported that *afp* gene expression, which takes place mainly in yolk sac and fetal liver, also occurs in normal human placenta, specifically in early pregnancy. The aim of the present study was to determine the precise location of AFP synthesis sites within the placental villi. In situ hybridization and immunohistochemical experiments were performed on sections obtained from placentas of first-trimester and full-term pregnancies. We found that the pattern of *afp* gene expression was restricted to specific villous trophoblastic areas in early placentas. Both *afp* transcripts and AFP protein were mainly located in discontinuous regions, at junctions between two villi and at budding sites. In contrast, no AFP expression was detected in the cytotrophoblastic extravillous proliferative zone or in other placental cell types. According to the earlier studies, no AFP synthesis was detected in placental villous tissue from full-term pregnancies, using in situ hybridization and immunohistochemistry.

FAYARD B., FAY N., DAVID G., DOUCET J., MELKI R.

Packing of the prion Ure2p in protein fibrils probed by fluorescence X-ray near-edge structure spectroscopy at sulfur K-edge.

J. Mol. Biol., 356 (4), 843-849, 2006

(Services cités : UPR 9078)

The soluble protein Ure2p from the yeast *Saccharomyces cerevisiae* assembles in vitro into straight and insoluble protein fibrils, through subtle changes of conformation. Whereas the structure of soluble Ure2p has been revealed by X-ray crystallography, further characterization of the structure of insoluble Ure2p fibrils is needed. We performed X-ray absorption near-edge spectroscopy (XANES) at the sulfur K-edge to probe the state of Cys221 in the fibrillar form of Ure2pC221 and provide structural information on the structure of Ure2p within fibrils. Although the Ure2p dimer dissociation into its constituent monomers has proven to be a prerequisite for assembly into fibrils, we showed the ability of every Ure2pC221 monomer to establish disulfide bonds upon incubation of the fibrils under oxidizing conditions. Our result indicates either that the constituent unit of the fibrillar form of the protein is a dimeric Ure2p or that the fibrils are made of protofilaments assembled in such a way that the residue C221 from a Ure2p molecule in one protofilament is located in the vicinity of a C221 residue from another molecule belonging to a neighbor protofilament.

HURTAUD C., GELLY C., BOUILLAUD F., LEVI-MEYRUEIS C.

Translation control of UCP2 synthesis by the upstream open reading frame.

Cell. Mol. Life Sci., 63 (15), 1780-1789, 2006

(Services cités : UPR 9078)

Uncoupling protein 2 (UCP2) belongs to a family of transporters of the mitochondrial inner membrane. In vivo low expression of UCP2 contrasts with a high UCP2 mRNA level, and induction of UCP2 expression occurs without change in mRNA level, demonstrating a translational control. The UCP2 mRNA is characterized by a long 5' untranslated region (5'UTR), in which an upstream open reading frame (uORF) codes for a 36-amino-acid sequence. The 5'UTR and uORF have an inhibitory role in the translation of UCP2. The present study demonstrates that the 3' region of the uORF is a major determinant for this inhibitory role. In this 3' region, a single-base substitution that kept the codon sense unchanged significantly modified UCP2 translation, whereas some important amino acid changes had no effect. We discuss our results within the framework of the existing models explaining initiation of translation downstream of a uORF.

MOZO J., FERRY G., MASSCHELEYN S., MIROUX B., BOUTIN J.A., BOUILLAUD F.

Assessment of a high-throughput screening methodology for the measurement of purified UCP1 uncoupling activity.

Anal. Biochem., 351 (2), 201-206, 2006

(Services cités : UPR 9078)

Three mitochondrial uncoupling proteins (UCP1, 2, 3) have been described. The proton transport activity of UCP1 triggers mitochondrial uncoupling and thermogenesis but the roles of UCP2 and UCP3 remain debated. Accordingly, compounds able to finely control the proton permeability of the mitochondrial inner membrane where and when needed may have enormous practical

consequences. Using purified hamster brown adipose tissue UCP1 reconstituted in liposomes, we describe herein a robust assay allowing the measurement of this artificial membrane conductance to protons in a format compatible with high-throughput screening. The assay was initially developed with a known chemical protonophore in an apoteic system. Then, using the proteolipid reconstituted UCP1 preparation, we assessed the assay with known modulators of UCP1, particularly retinoic acid and guanosine 5'-triphosphate. The system was developed for a 96-well plate format. We then exemplified its use by generating primary data on a set of compounds screened in this system. These primary data will open new routes for the search of candidate compounds that will help biochemical studies on UCPs.

MOZO J., FERRY G., STUDENY A., PECQUEUR C., RODRIGUEZ M., BOUTIN J.A., BOUILLAUD F.

Expression of UCP3 in CHO cells does not cause uncoupling, but controls mitochondrial activity in the presence of glucose.

Biochem. J., 393 (Pt 1), 431-439, 2006

(Services cités : [UPR 9078](#))

The proton-transport activity of UCP1 (uncoupling protein 1) triggers mitochondrial uncoupling and thermogenesis. The exact role of its close homologues, UCP2 and UCP3, is unclear. Mounting evidence associates them with the control of mitochondrial superoxide production. Using CHO (Chinese-hamster ovary) cells stably expressing UCP3 or UCP1, we found no evidence for respiration uncoupling. The explanation lies in the absence of an appropriate activator of UCP protonophoric function. Accordingly, the addition of retinoic acid uncouples the respiration of the UCP1-expressing clone, but not that of the UCP3-expressing ones. In a glucose-containing medium, the extent of the hyperpolarization of mitochondria by oligomycin was close to 22 mV in the five UCP3-expressing clones, contrasting with the variable values observed with the 15 controls. Our observations suggest that, when glycolysis and mitochondria generate ATP, and in the absence of appropriate activators of proton transport, UCPs do not transport protons (uncoupling), but rather other ions of physiological relevance that control mitochondrial activity. A model is proposed using the known passive transport of pyruvate by UCP1.

NUBEL T., RICQUIER D.

Respiration under control of uncoupling proteins: Clinical perspective.

Hormone Res., 65 (6), 300-310, 2006

(Services cités : [UPR 9078](#))

The term 'uncoupling protein' was originally used for the mitochondrial membrane protein UCP1, which is uniquely present in mitochondria of brown adipocytes, thermogenic cells that regulate body temperature in small rodents, hibernators and mammalian newborns. In these cells, UCP1 acts as a proton carrier activated by free fatty acids and creates a shunt between complexes of the respiratory chain and ATP-synthase resulting in a futile proton cycling and dissipation of oxidation energy as heat. Recent identification of new homologues to UCP1 expressed in brown and white adipose tissue, muscle, brain and other tissues together with the hypothesis that these novel uncoupling proteins (UCPs) may regulate thermogenesis and/or fatty acid metabolism and furthermore may protect against free radical oxygen species production have generated considerable optimism for rapid advances in the identification of new targets for pharmacological

management of complex pathological syndromes such as obesity, type 2 diabetes or chronic inflammatory diseases. However, since the physiological and biochemical roles of the novel UCPs are not yet clear, the main challenge today consists first of all in providing mechanistic explanation for their functions in cellular physiology. This lively awaited information may be the basis for potential pharmacological targeting of the UCPs in future.

RICQUIER D.

Fundamental mechanisms of thermogenesis.

C. R. Biol., 329 (8), 578-586, 2006

(Services cités : UPR 9078)

Thermogenesis is an obligatory consequence of cellular metabolism and is identified as a unique property of homeotherms which have to maintain constant their body temperature in a cold environment. Physiologically, thermogenesis is made of basal metabolism, post-prandial thermogenesis, exercise-induced thermogenesis and adaptive thermogenesis induced by changes in the environmental temperature. Biochemically, thermogenesis comes from exergonic reactions from a loose coupling between endergonic and exergonic reactions. In cells, respiration and oxidations occur in mitochondria which ensure the coupling of oxidative energy to ATP synthesis. Identification of mitochondrial uncoupling proteins UCP allowed further understanding of the mechanism of coupling or uncoupling of respiration to ADP phosphorylation. Such data maybe of help in the understanding, or possible treatment, of certain types of obesity. To cite this article: D. Ricquier, *C. R. Biologies* 329 (2006).

RICQUIER D., BASDEVANT A.

Diseases of the adipose tissue.

C. R. Biol., 329 (8), 559-561, 2006

(Services cités : UPR 9078)

ROUSSET S., EMRE Y., JOIN-LAMBERT O., HURTAUD C., RICQUIER D., CASSARD-DOULCIER A.M.

The uncoupling protein 2 modulates the cytokine balance in innate immunity.

Cytokine, 35 (3-4), 135-142, 2006

(Services cités : UPR 9078, U570)

The uncoupling protein 2 (UCP2) is located in the inner mitochondrial membrane and downregulates the production of reactive oxygen species (ROS). Recent data suggested a role for UCP2 in the immune response. We analyzed further this hypothesis during acute *Listeria monocytogenes* infection in mice. Death of infected Ucp2(-/-) mice was delayed in comparison with Ucp2(+/+), suggesting a role of UCP2 in the early step of the immune response. In vitro, the higher resistance of Ucp2(-/-) mice was not associated with a better control of bacterial growth by macrophages. In vivo, a significant increase of recruited phagocytes was observed in the spleen of Ucp2(-/-) mice. This was associated with a higher level of ROS in the spleen. Upregulation of pro-inflammatory cytokines IFN γ , IL6, and IL1 β and of the chemokine MCP1 was observed in Ucp2(-/-) mice 4 days after infection, preceded by a decrease of the anti-inflammatory cytokine IL10 production. Present data highlight that, in an acute model of

infection, UCP2 modulates innate immunity, via the modulation of ROS production, cytokine and chemokine production and consequently phagocyte recruitment.

VIEIRA V., DAVID G., ROCHE O., de LA HOUSSAYE G., BOUTBOUL S., ARBOGAST L., KOBETZ A., ORSSAUD C., CAMAND O., SCHORDERET D.F., MUNIER F., ROSSI A., DELEZOIDE A.L., MARSAC C., RICQUIER D., DUFIER J.L., MENASCHE M., ABITBOL M.

Identification of four new PITX2 gene mutations in patients with Axenfeld-Rieger syndrome.

Mol. Vis., 12 (.), 1448-1460, 2006

(Services cités : CERTO, Ophtalmologie, UPR 9078)

PURPOSE: Axenfeld Rieger syndrome (ARS) is an autosomal dominant inherited disorder affecting development of the ocular anterior chamber, abdomen, teeth and facial structures. The PITX2 gene is a major gene encoding a major transcription factor associated with ARS.

METHODS: ARS patients were collected from six unrelated families. Patients and their families were ophthalmologically phenotyped and their blood was collected for DNA extraction. We screened the coding region of human PITX2 gene by direct sequencing. The consequences of the mutations described were investigated by generating crystallographic representations of the amino acid changes. In order to better understand the occurrence of glaucoma in ARS patients, we studied the PITX2 gene expression in human embryonic and fetal ocular tissue sections.

RESULTS: We identified four novel PITX2 genetic alterations in four unrelated families with ARS. These mutations included two nonsense mutations (E55X and Y121X), an eight nucleotides insertion (1251 ins CGACTCCT) and a substitution (F58L), in familial and sporadic cases of ARS. We also showed for the first time that PITX2 is expressed at early stages of the human embryonic and fetal periocular mesenchyme, as well as at later stages of human development in the fetal ciliary body, ciliary processes, irido corneal angle and corneal endothelium. The human fetal eye PITX2 gene expression pattern reported here for the first time provides a strong basis for explaining the frequent occurrence of glaucoma in patients affected by PITX2 gene mutations. **CONCLUSIONS:** Two mutations identified affect the homeodomain (E55X and F58L). The E55X nonsense mutation is likely to alter dramatically the DNA-binding capabilities of the PITX2 homeodomain. Furthermore, there is a complete loss of the carboxy-terminal part of the PITX2 protein beyond the site of the mutation. The phenylalanine F58 is known to contribute to the hydrophobic network of the homeodomain. The crystallographic representations of the mutation F58L show that this mutation may change the conformation of the helical core. The F58L mutation is very likely to modify the homeodomain conformation and probably alters the DNA binding properties of PITX2. The other mutations (Y121X and the eight-nucleotide insertion (1251 ins CGA CTC CT) CGA CTC CT, at position 224 in PITX2A) result in partial loss of the C-terminal domain of PITX2. Pitx2 synergistically transactivates the prolactin promoter in the presence of the POU homeodomain protein Pit-1. Pitx2 activity is regulated by its own C-terminal tail. This region contains a highly conserved 14-amino-acid element involved in protein-protein interactions. The C-terminal 39-amino-acid tail represses DNA binding activity and is required for Pitx2 interactions with other transcription factors, for Pitx2-Pit-1 interaction and Pit-1 synergism. Pit-1 interaction with the Pitx2 C terminus masks the inhibitory effect and promotes increased DNA binding activity. Thus, the partial or complete loss of the C terminus tail can lead to decreased or absent DNA binding activity and trigger severe ARS phenotypes. Our in situ hybridization results obtained on human embryonic and fetal ocular tissue sections constitute the first molecular histological data providing an explanation for the

occurrence of precocious glaucoma in human patients affected by ARS caused by PITX2 mutations. Further structural and biochemical studies are needed for understanding the wide spectrum of clinical phenotypes caused by the increasing number of new PITX2 mutations found in ARS affected patients.

VOGLER S., PAHNKE J., ROUSSET S., RICQUIER D., MOCH H., MIROUX B., IBRAHIM S.M.

Uncoupling Protein 2 Has Protective Function during Experimental Autoimmune Encephalomyelitis.

Amer. J. Pathol., 168 (5), 1570-1575, 2006

(Services cités : [UPR 9078](#))

Uncoupling protein 2 (UCP2) is a member of the mitochondrial transporter superfamily that is expressed in many tissues, including immune cells. UCP2 prevents oxidative stress by reducing reactive oxygen species. Using UCP2-deficient mice, it was shown that UCP2 is involved in the regulation of insulin secretion, in the resistance to infection, and in atherosclerosis. Here, we investigated the role of UCP2 in experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis. Immunized C57BL/6J UCP2-deficient mice showed a slightly delayed onset during experimental autoimmune encephalomyelitis (13.0 +/- 0.6 versus 11.5 +/- 0.8 in wild-type controls) and developed significantly higher disease scores than littermate controls (maximum disease score of 2.9 +/- 0.2 versus 1.7 +/- 0.2, P = 0.001). Higher levels of infiltrating T cells into the spinal cord meninges and parenchyma were observed. The T-cell proliferative response to the specific antigen was increased in UCP2-deficient mice compared with littermate controls, and CD4 cells of UCP2 knockout mice produced significantly higher levels of pro-inflammatory cytokines, eg, tumor necrosis factor-alpha and interleukin-2, resulting from a Th1 response. Mice lacking UCP2 also developed a higher B-cell response. Concomitantly, CD4 and CD8 cells of the UCP2-deficient mice showed increased production of reactive oxygen species. These results suggest a protective function of UCP2 in chronic inflammatory diseases such as multiple sclerosis.

2005

CHAUVET C., BOIS-JOYEUX B., FONTAINE C., GERVOIS P., BERNARD M.A., STAELS B., DANAN J.L.

The Gene Encoding Fibrinogen-beta Is a Target for Retinoic Acid Receptor-Related Orphan Receptor alpha.

Mol. Endocrinol., 19 (10), 2517-2526, 2005

(Services cités : [UPR 9078](#))

Fibrinogen is a plasma protein synthesized by the liver. It is composed of three chains (alpha, beta, gamma). In addition to its main function as a coagulation factor, this acute phase protein is also a risk marker for atherosclerosis. Retinoic acid receptor-related orphan receptor (ROR)alpha is a nuclear receptor modulating physiopathological processes such as cerebellar ataxia, inflammation, atherosclerosis, and angiogenesis. In this study, we identified RORalpha as a regulator of fibrinogen-beta gene expression in human hepatoma cells and in mouse liver. A putative RORalpha response element (RORE) was identified in the human fibrinogen-beta promoter. EMSA showed that RORalpha binds specifically to this RORE, and cotransfection experiments in HepG2 hepatoma cells indicated that this RORE confers RORalpha-dependent

transcriptional activation to both the human fibrinogen-beta and the thymidine kinase promoters. Stable transfection experiments in HepG2 and Hep3B hepatoma cells demonstrated that overexpression of RORalpha specifically increases endogenous fibrinogen-beta mRNA levels. Chromatin immunoprecipitation experiments revealed that the fibrinogen-beta RORE is occupied by RORalpha in HepG2 cells. Thus, the human fibrinogen-beta gene is a direct target for RORalpha. Furthermore, fibrinogen-beta mRNA levels in liver and plasma fibrinogen concentrations are specifically decreased in staggerer mice, which are homozygous for a deletion invalidating the Rora gene. Taken together, these data add further evidence for an important role of RORalpha in the control of liver gene expression with potential pathophysiological consequences on coagulation and cardiovascular risk.

CRISCUOLO F., CHASTEL O., BERTILE F., GABRIELSEN G.W., LE MAHO Y., RACLOT T.

Corticosterone alone does not trigger a short term behavioural shift in incubating female common eiders *Somateria mollissima*, but does modify long term reproductive success.

J. Avian Biol., 36 (4), 306-312, 2005

(Services cités : UPR 9078)

The trade-off between reproductive effort and adult survival in birds is modulated by several factors. Corticosterone and prolactin have additive effects on reproductive behaviour by stimulating foraging and parental behaviours, respectively. When incubation is associated with fasting, nest desertion is supposed to be activated by an unknown refeeding signal when body condition becomes critically deteriorated. The concomitant rise in corticosterone levels has been suggested to be the triggering factor. We tested the role of corticosterone on reproductive success by observing the effect of corticosterone implants on reproductive success and on plasma prolactin concentration in female common eiders *Somateria mollissima*. Implanted females showed a significant increase in corticosterone and a decrease in prolactin levels. Despite their enhanced daily body mass loss, females did not abandon incubation nor did they start to refeed in the four days following implantation. These data show that the experimentally induced rise in plasma corticosterone concentration alone does not trigger nest desertion. However, after 25 days of incubation, implanted females displayed a higher rate of egg loss, suggesting lower nest attentiveness towards the end of incubation. We suggest that the short-term effects of corticosterone may be dependent on the energy state of the bird. However, the late-induced change in reproductive success is indirectly linked to corticosterone, and we suggest that either a prolactin decrease, or a depletion in protein body reserves, may participate in the long-term adjustment of incubation behaviour in female eiders.

CRISCUOLO F., GONZALEZ-BARROSO-MADRID M.D.M., BOUILLAUD F., RICQUIER D., MIROUX B., SORCI G.

Mitochondrial Uncoupling Proteins: New Perspectives for Evolutionary Ecologists.

Am. Nat., 166 (6), 686-699, 2005

(Services cités : UPR 9078)

Reactive oxygen species (ROS)-induced damage on host cells and molecules has been considered the most likely proximal mechanism responsible for the age-related decline in organismal performance. Organisms have two possible ways to reduce the negative effect of ROS: disposing of effective antioxidant defenses and minimizing ROS production. The unbalance between the

amount of ROS produced and the availability of antioxidant defenses determines the intensity of so-called oxidative stress. Interestingly, most studies that deal with the effect of oxidative stress on organismal performance have focused on the antioxidant defense compartment and, surprisingly, have neglected the mechanisms that control ROS production within mitochondria. Uncoupling proteins (UCPs), mitochondrial transporters of the inner membrane, are involved in the control of redox state of cells and in the production of mitochondrial ROS. Given their function, UCPs might therefore represent a major mechanistic link between metabolic activity and fitness. We suggest that by exploring the role of expression and function of UCPs both in experimental as well as in comparative studies, evolutionary biologists may gain better insight into this link.

CRISCUOLO F., GONZALEZ-BARROSO-MADRID M.D.M., MAHO Y.L., RICQUIER D., BOUILLAUD F.

Avian uncoupling protein expressed in yeast mitochondria prevents endogenous free radical damage.

Proc. Roy Soc. Lond. [Biol], 272 (1565), 803-810, 2005

(Services cités : [UPR 9078](#))

The longevity of birds is surprising since they exhibit high metabolic rates and elevated blood sugar levels compared with mammals of the same body size, which presumably expose them to higher rates of free oxygen radical production, which is implicated in accelerated senescence. Uncoupling proteins (UCPs) are transporters of the inner mitochondrial membrane and their physiological activity is still a subject of debate. Avian UCP was found in birds but data on its activity are scarce. Avian UCP (*Gallus gallus*) was overexpressed in yeast and we assessed its ability to prevent mitochondrial reactive oxygen species (ROS) production by measuring ROS damage (aconitase activity) and antioxidant defences (MnSOD activity). We show that avian UCP protects yeast mitochondria against the deleterious impact of ROS, but without stimulation of superoxide dismutase activity. Avian UCP protein was specifically immunodetected and retinoic acid, which belongs to the carotenoid family, was found to trigger its activity. These data show that avian UCP basal activity protects against ROS damage. However, when activated by retinoic acid, avian UCP can also operate as the mammalian thermogenic UCP1. The hypothesis that avian UCP activities are state- and species-dependent is further discussed.

GNANALINGHAM M.G., MOSTYN A., WANG J., WEBB R., KEISLER D.H., RAVEN N., ALVES-GUERRA M.C., PECQUEUR C., MIROUX B., STEPHENSON T., SYMONDS M.E.

Tissue-specific effects of leptin administration on the abundance of mitochondrial proteins during neonatal development.

J. Endocrinol., 187 (1), 81-88, 2005

(Services cités : [UPR 9078](#))

Many tissues undergo a rapid transition after birth, accompanied by dramatic changes in mitochondrial protein function. In particular, uncoupling protein (UCP) abundance increases at birth in the lung and adipose tissue, to then gradually decline, an adaptation that is important in enabling normal tissue function. Leptin potentially mediates some of these changes and is known to promote the loss of UCP1 from brown fat but its effects on UCP2 and related mitochondrial proteins (i.e. voltage-dependent anion channel (VDAC) and cytochrome c) in other tissues are

unknown. We therefore determined the effects of once-daily jugular venous administration of ovine recombinant leptin on mitochondrial protein abundance as determined by immunoblotting in tissues that do (i.e. the brain and pancreas) and do not (i.e. liver and skeletal muscle) express UCP2. Eight pairs of 1-day-old lambs received either 100 mug leptin or vehicle daily for 6 days, before tissue sampling on day 7. Administration of leptin diminished UCP2 abundance in the pancreas, but not the brain. Leptin administration had no effect on the abundance of VDAC or cytochrome c in any tissue examined. In leptin-administered animals, but not controls, UCP2 abundance in the pancreas was positively correlated with VDAC and cytochrome c content, and UCP2 abundance in the brain with colonic temperature. In conclusion, leptin administration to neonatal lambs causes a tissue-specific loss of UCP2 from the pancreas. These effects may be important in the regulation of neonatal tissue development and potentially for optimising metabolic control mechanisms in later life.

GNANALINGHAM M.G., MOSTYN A., WEBB R., KEISLER D.H., RAVER N., ALVES-GUERRA M.C., PECQUEUR C., MIROUX B., SYMONDS M.E., STEPHENSON T.

Differential effects of leptin administration on the abundance of UCP2 and glucocorticoid action during neonatal development.

Amer. J. Physiol. - Endocrinol. Met., 289 (6), E1093-E1100, 2005

(Services cités : UPR 9078)

In the neonate, adipose tissue and the lung both undergo a rapid transition after birth, which results in dramatic changes in uncoupling protein abundance and glucocorticoid action. Leptin potentially mediates some of these adaptations and is known to promote the loss of uncoupling protein (UCP)1, but its effects on other mitochondrial proteins or glucocorticoid action are not known. We therefore determined the effects of acute and chronic administration of ovine recombinant leptin on brown adipose tissue (BAT) and/or lung in neonatal sheep. For the acute study, eight pairs of 1-day-old lambs received, sequentially, 10, 100, and 100 mug of leptin or vehicle before tissue sampling 4 h from the start of the study, whereas in the chronic study, nine pairs of 1-day-old lambs received 100 mug of leptin or vehicle daily for 6 days before tissue sampling on day 7. Acute leptin decreased the abundance of UCP2, glucocorticoid receptor, and 11beta-hydroxysteroid dehydrogenase (11beta-HSD) type 1 mRNA and increased 11beta-HSD type 2 mRNA abundance in BAT, a pattern that was reversed with chronic leptin administration, which also diminished lung UCP2 protein abundance. In BAT, UCP2 mRNA abundance was positively correlated to plasma leptin and nonesterified fatty acids and negatively correlated to mean colonic temperature in the leptin group at 7 days. In conclusion, leptin administration to the neonatal lambs causes differential effects on UCP2 abundance in BAT and lung. These effects may be important in the development of these tissues, thereby optimizing lung function and fat growth.

HAGUENAUER A., RAIMBAULT S., MASSCHELEYN S., GONZALEZ-BARROSO-MADRID M.D.M., CRISCUOLO F., PLAMONDON J., MIROUX B., RICQUIER D., RICHARD D., BOUILLAUD F., PECQUEUR C.

A New Renal Mitochondrial Carrier, KMCP1, Is Up-regulated during Tubular Cell Regeneration and Induction of Antioxidant Enzymes.

J. Biol. Chem., 280 (23), 22036-22043, 2005

(Services cités : UPR 9078)

The mitochondrial carrier family transports a variety of metabolites across the inner mitochondrial membrane. We identified and cloned a new member of this family, KMCP1 (kidney mitochondrial carrier protein-1), that is highly homologous to the previously identified protein BMCP1 (brain mitochondrial carrier protein-1). Western blotting and in situ experiments showed that this carrier is expressed predominantly within the kidney cortex in the proximal and distal tubules. KMCP1 was increased during fasting and during the regenerative phase of glycerol-induced renal failure. We show that both situations are associated with transiently increased expression of superoxide-generating enzymes, followed by increased mitochondrial metabolism and antioxidant defenses. Given that KMCP1 expression occurs simultaneously with these latter events, we propose that KMCP1 is involved in situations in which mitochondrial metabolism is increased, in particular when the cellular redox balance tends toward a pro-oxidant status.

LESCELLE X., GOUBERN M., ANDRIAMIHAJA M., BLOTTIERE H.M., COUPLAN E., GONZALEZ-BARROSO-MADRID M.D.M., PETIT C., PAGNIEZ A., CHAUMONTET C., MIGNOTTE B., BOUILLAUD F., BLACHIER F.

Adaptative metabolic response of human colonic epithelial cells to the adverse effects of the luminal compound sulfide.

Biochim. Biophys. Acta - Gen. Subjects, 1725 (2), 201-212, 2005

(Services cités : UPR 9078)

Hydrogen sulfide (H₂S), a bacterial metabolite present in the lumen of the large intestine, is able to exert deleterious effects on the colonic epithelium. The mechanisms involved are still poorly understood, the reported effect of sulfide being its capacity to reduce n-butyrate beta-oxidation in colonocytes. In this work, we studied both the acute effect of the sodium salt of H₂S on human colonic epithelial cell metabolism and the adaptative response of these cells to the pre-treatment with this agent. Using the human colon carcinoma epithelial HT-29 Glc(-/+) cell model, we found that the acute effect of millimolar concentrations of NaHS was to inhibit l-glutamine, n-butyrate and acetate oxidation in a dose-dependent manner. Using micromolar concentrations of NaHS, a comparable effect but largely reversible was observed for O₂ consumption and cytochrome c oxidase activity. Pre-treatment with 1 mM NaHS induced several adaptative responses. Firstly, increased lactate release and decreased cellular oxygen consumption evidenced a Pasteur-like effect which only partly compensated for the altered mitochondrial ATP production. Thus, a decrease in the proliferation rate with a constant adenylate charge was observed. Secondly, in these pre-treated cells, NaHS induced a hypoxia-like effect on cytochrome c oxidase subunits I and II which were decreased. Thirdly, a mild uncoupling of mitochondrial respiration possibly resulting from an increase of UCP 2 protein was observed. The NaHS antimetabolic activity was not due to cellular apoptosis and/or necrosis but to a proportional slowdown in all cell cycle phases. These results are compatible with a metabolic adaptative response of the HT-29 colonic epithelial cells to sulfide-induced O₂ consumption reduction which, through the maintenance of a constant energetic load and an increased mitochondrial proton leak, would participate in the preservation of cellular viability.

MONDON F., MIGNOT T.M., REBOURCET R., JAMMES H., DANAN J.L., FERRE F., VAIMAN D.

Profiling of oxygen-modulated gene expression in early human placenta by systematic

sequencing of suppressive subtractive hybridization products.

Physiol. Genomics, 22 (1), 99-107, 2005

(Services cités : UPR 9078)

Villi from first-trimester human placenta were exposed to oxygen concentrations of either 2 or 20% during 3 h to construct two reciprocally subtracted libraries using the suppressive subtractive hybridization (SSH) methodology. After cloning, sequencing, and gene identification, the genes (1,071 clones corresponding to 822 different sequences) were classified according to 1) the subtracted library from which they originated and 2) within 58 groups of gene functions. We then developed a logarithm of the odds (LOD) test to identify a possible excess of genes in each group. We show that genes involved in angiogenesis are significantly overrepresented in the "hypoxic" condition (2% O₂), whereas apoptotic genes are overrepresented in the "normoxic" condition (20% O₂). Furthermore, we observed an excess of kinases relative to phosphatases and an excess of genes involved in proliferation over genes involved in cell growth in the hypoxic condition. To validate our results, we used quantitative RT-PCR to analyze the set of eight genes involved in angiogenesis on six independent placentas. Finally, we studied the distribution of gene clusters on human chromosomes to check whether their chromosomal distribution was random or not. We observed on human chromosome 11 a clear clustering of genes regulated similarly by O₂ tension, and we also discovered indications that such clustering exists on chromosomes 6 and 12.

MOZO J., EMRE Y., BOUILLAUD F., RICQUIER D., CRISCUOLO F.

Thermoregulation: What Role for UCPs in Mammals and Birds?

Biosci. Rep., 25 (3-4), 227-249, 2005

(Services cités : UPR 9078)

Mammals and birds are endotherms and respond to cold exposure by the means of regulatory thermogenesis, either shivering or non-shivering. In this latter case, waste of cell energy as heat can be achieved by uncoupling of mitochondrial respiration. Uncoupling proteins, which belong to the mitochondrial carrier family, are able to transport protons and thus may assume a thermogenic function. The mammalian UCP1 physiological function is now well understood and gives to the brown adipose tissue the capacity for heat generation. But is it really the case for its more recently discovered isoforms UCP2 and UCP3? Additionally, whereas more and more evidence suggests that non-shivering also exists in birds, is the avian UCP also involved in response to cold exposure? In this review, we consider the latest advances in the field of UCP biology and present putative functions for UCP1 homologues.

NEDERGAARD J., RICQUIER D., KOZAK L.P.

Uncoupling proteins: current status and therapeutic prospects.

EMBO Rep., 6 (10), 917-921, 2005

(Services cités : UPR 9078)

PETIT C., PIETRI-ROUXEL F., LESNE A., LESTE-LASSERRE T., MATHEZ D., NAVIAUX R.K., SONIGO P., BOUILLAUD F., LEIBOWITCH J.

Oxygen consumption by cultured human cells is impaired by a nucleoside analogue cocktail that

inhibits mitochondrial DNA synthesis.

Mitochondrion, 5 (3), 154-161, 2005

(Services cités : [UPR 9078](#))

We evaluated oxygen consumption rates in human cells cultured in the presence of a nucleoside analog reverse transcriptase inhibitor (NRTI) cocktail that inhibits mitochondrial DNA synthesis. We treated a proliferating human lymphocyte cell line and a primary culture of human adipose cells with antiretroviral drugs (AZT+ddC+d4T). The effects of these drugs on mitochondrial DNA (mtDNA) levels and oxygen consumption rates were evaluated using semi-quantitative real-time PCR and an on-line monitoring Clark electrode system. We found that the NRTI treatment lowered oxygen consumption rates and inhibited mitochondrial DNA replication in human cell cultures. Inhibition of oxygen consumption was linearly proportional to inhibition of mtDNA replication. These results show for the first time that mitochondrial respiration is impaired in NRTI sensitive cells. The linear relationship between NRTI inhibition of respiration and NRTI inhibition of mtDNA replication indicates that small decreases in mtDNA levels can lead to respiratory deficits in the tissues of patients treated with anti-HIV drugs. We propose a model that takes into account the small differences in metabolic dynamics between peripheral and axial/visceral fat tissues. This model explains how NRTI-related respiratory deficits may lead to the presentation of opposing lipodystrophic syndromes in same patient.

RICQUIER D.

Respiration uncoupling and metabolism in the control of energy expenditure.

Proc. Nutr. Soc., 64 (1), 47-52, 2005

(Services cités : [UPR 9078](#))

Metabolic energy expenditure negatively regulates energy balance. Metabolic and catabolic pathways contribute to energy expenditure. Catabolic pathways split C-containing molecules into small molecules and generate reduced coenzymes and ATP. For a given amount of substrate, any increase in energy expenditure requires either increased ATP hydrolysis or decreased ATP synthesis. In skeletal muscles substrate utilisation is coupled to ATP production, whereas ATP hydrolysis is activated during physical exercise and increases energy expenditure. In brown adipose tissue activation of cells during exposure to cold increases substrate utilisation in such a way that glucose and fatty acid oxidation detach from the orthodox coupling to ATP synthesis and result in thermogenesis. The unique mechanism of uncoupling respiration that occurs in brown adipocyte mitochondria represents an attractive strategy for promoting energy expenditure and decreasing the fat content of the body. Moreover, ectopic expression of brown fat uncoupling protein (UCP) 1 in mouse skeletal muscle and induction of UCP1 in mouse or human white adipocytes promote fatty acid oxidation and resistance to obesity. In normal conditions UCP2 and UCP3 do not seem to contribute substantially to energy expenditure. Whether the induction of UCP1, the induction of other UCP or chemical mild uncoupling represent promising strategies for attenuating nutrient efficiency and counteracting obesity should be considered.

SABAA N., MIROUX B., COFFMAN T.M., COLLINS S., RICQUIER D., DUSSAULE J.C., THARAUX P.L.

UCP-2 does not modulate angiotensin II-induced high blood pressure but limits the development of hypertensive renal sclerosis.

J. Hypertension, 23 (8), A11, 2005

(Services cités : UPR 9078)

SABAA N., MIROUX B., COFFMAN T.M., COLLINS S., RICQUIER D., DUSSAULE J.C., THARAUX P.L.

UCP-2 does not modulate angiotensin II-induced high blood pressure but limits the development of hypertensive renal sclerosis.

Arch. Mal. Coeur Vaisseaux, 98 (7-8), 850-851, 2005

(Services cités : UPR 9078)

TABARIN A., CHAVES Y.D., CARMONA MDEL C., CATARGI B., ZORRILLA E.P., ROBERTS A.J., COSCINA D.V., ROUSSET S., REDONNET A., PARKER G.C., INOUE K., RICQUIER D., PENICAUD L., KIEFFER B.L., KOOB G.F.

Resistance to Diet-Induced Obesity in micro-Opioid Receptor-Deficient Mice: Evidence for a "Thrifty Gene".

Diabetes, 54 (12), 3510-3516, 2005

(Services cités : UPR 9078)

Using pharmacological tools, a role for opioid receptors in the regulation of food intake has been documented. However, the involvement of specific receptor subtypes remains questionable, and little information is available regarding a role for opioid receptors in energy metabolism. Using adult male mice lacking the mu-opioid receptor (MOR) gene (MOR(-/-)), we show that the MOR is not essential for the maintenance of normal levels of ad libitum food intake but does modulate the efficiency of energy storage during high-fat diets through the regulation of energy partitioning. When fed a regular diet, MOR(-/-) mice displayed only subtle alterations in energy homeostasis, suggesting a relative overuse of fat as a fuel source in the fed state. When fed a high-fat diet, MOR(-/-) mice were resistant to obesity and impaired glucose tolerance, despite having similar energy intake to wild-type mice. This resistance to obesity was associated with a strong induction of the expression of key mitochondrial enzymes involved in fatty acid oxidation within skeletal muscle. This metabolic role of the MOR, which is consistent with the properties of a "thrifty gene," suggests that the MOR pathway is a potential target for pharmacological intervention in the treatment of obesity associated with the intake of fatty diets.

2004

CHAUVET C., BOIS-JOYEUX B., BERRA E., POUYSSEGUR J., DANAN J.L.

The gene encoding human retinoic acid-receptor-related orphan receptor alpha is a target for hypoxia-inducible factor 1.

Biochem. J., 384 (Pt 1), 79-85, 2004

(Services cités : UPR 9078, IRNEM)

Retinoic acid-receptor-related orphan receptor (ROR) alpha is a nuclear receptor involved in many pathophysiological processes such as cerebellar ataxia, inflammation, atherosclerosis and angiogenesis. In the present study we first demonstrate that hypoxia increases the amount of Rora transcripts in a wide panel of cell lines derived from diverse tissues. In addition, we identified a functional promoter sequence upstream of the first exon of the human Rora gene, spanning -487

and -45 from the translation initiation site of RORalpha1. When cloned in a luciferase reporter vector, this sequence allowed the efficient transcription of the luciferase gene in several cell lines. Interestingly, the activity of the Rora promoter was enhanced by hypoxia in HepG2 human hepatoma cells, and this effect was dependent on an HRE (hypoxia response element) spanning from -229 to -225. Using electrophoretic-mobility-shift assays, we showed that HIF-1 (hypoxia-inducible factor 1), which plays a key role in the transcriptional response to hypoxia, bound to this HRE. Overexpression of HIF-1alpha increased the activity of the Rora promoter through the HRE. Overexpression of a dominant-negative form of HIF-1alpha producing transcriptionally inactive HIF-1alpha/HIF-1beta dimers abolished hypoxic activation of the Rora promoter. This indicated that HIF-1 is involved in the response of RORalpha to hypoxia. Taken together, our data reveal Rora as a new HIF-1 target gene. This illustrates, at the molecular level, the existence of cross-talk between signalling pathways mediated by HIF-1 and those mediated by nuclear receptors.

DE BILBAO F., ARSENIJEVIC D., VALLET P., HJELLE O.P., OTTERSEN O.P., BOURAS C., RAFFIN Y., ABOU K., LANGHANS W., COLLINS S., PLAMONDON J., ALVES-GUERRA M.C., HAGUENAUER A., GARCIA I., RICHARD D., RICQUIER D., GIANNAKOPOULOS P.

Resistance to cerebral ischemic injury in UCP2 knockout mice: evidence for a role of UCP2 as a regulator of mitochondrial glutathione levels.

J. Neurochem., 89 (5), 1283-1292, 2004

(Services cités : [UPR 9078](#))

Abstract Uncoupling protein 2 (UCP2) is suggested to be a regulator of reactive oxygen species production in mitochondria. We performed a detailed study of brain injury, including regional and cellular distribution of UCP2 mRNA, as well as measures of oxidative stress markers following permanent middle cerebral artery occlusion in UCP2 knockout (KO) and wild-type (WT) mice. Three days post ischemia, there was a massive induction of UCP2 mRNA confined to microglia in the peri-infarct area of WT mice. KO mice were less sensitive to ischemia as assessed by reduced brain infarct size, decreased densities of deoxyuridine triphosphate nick end-labelling (TUNEL)-labelled cells in the peri-infarct area and lower levels of lipid peroxidation compared with WT mice. This resistance may be related to the substantial increase of basal manganese superoxide dismutase levels in neurons of KO mice. Importantly, we found a specific decrease of mitochondrial glutathione (GSH) levels in UCP2 expressing microglia of WT, but not in KO mice after ischemia. This specific association between UCP2 and mitochondrial GSH levels regulation was further confirmed using lipopolysaccharide models of peripheral inflammation, and in purified peritoneal macrophages. Moreover, our data imply that UCP2 is not directly involved in the regulation of ROS production but acts by regulating mitochondrial GSH levels in microglia.

HOERTER J., GONZALEZ-BARROSO-MADRID M.D.M., COUPLAN E., MATEO P., GELLY C., CASSARD-DOULCIER A.M., DIOLEZ P., BOUILLAUD F.

Mitochondrial uncoupling protein 1 expressed in the heart of transgenic mice protects against ischemic-reperfusion damage.

Circulation, 110 (5), 528-533, 2004

(Services cités : [UPR 9078](#))

BACKGROUND: Mitochondrial respiration is the main source of energy in aerobic animal cells and is adapted to the energy demand by respiratory coupling. Uncoupling proteins (UCPs) perturb respiratory coupling by inducing a proton leak through the mitochondrial inner membrane. Although this could lead to deleterious energy waste, it may prevent the production of oxygen radicals when the rate of phosphorylation of ADP into ATP is low, whereas oxygen and substrate availability to mitochondria is high. The latter conditions are encountered during cardiac reperfusion after ischemia and are highly relevant to heart infarction. **METHODS AND RESULTS:** Heart function of 6 transgenic mice expressing high amounts of UCP1 and of 6 littermate controls was compared in isolated perfused hearts in normoxia, after 40-minute global ischemia, and on reperfusion. In normoxia, oxygen consumption, contractility (quantified as the rate-pressure product), and their relationship (energetic yield) were similar in controls and transgenic mice. Although UCP1 expression did not alter the sensitivity to ischemia, it significantly improved functional recovery on reperfusion. After 60 minutes of reperfusion, contractility was 2-fold higher in transgenic mice than in controls. Oxygen consumption remained significantly depressed in controls (53±27% of control), whereas it recovered strikingly to preischemic values in transgenic mice, showing uncoupling of respiration by UCP1 activity. Glutathione and aconitase, markers of oxidative damage, indicated lower oxidative stress in transgenic mice. **CONCLUSIONS:** UCP1 activity is low under normoxia but is induced during ischemia-reperfusion. The presence of UCP1 mitigates reperfusion-induced damage, probably because it lowers mitochondrial hyperpolarization at reperfusion.

MOSTYN A., LITTEN J.C., PERKINS K.S., ALVES-GUERRA M.C., PECQUEUR C., MIROUX B., SYMONDS M.E., CLARKE L.

Influence of genotype on the differential ontogeny of uncoupling protein 2 and 3 in subcutaneous adipose tissue and muscle in neonatal pigs.

J. Endocrinol., 183 (1), 121-131, 2004

(Services cités : [UPR 9078](#))

The present study aimed to determine whether porcine genotype and/or postnatal age influenced mRNA abundance or protein expression of uncoupling protein (UCP)2 or 3 in subcutaneous adipose tissue (AT) and skeletal muscle (SM) and the extent to which these differences are associated with breed-specific discordance in endocrine and metabolic profiles. Piglets from commercial and Meishan litters were ranked according to birth weight. Tissue samples were obtained from the three median piglets from each litter on either day 0, 4, 7, 14 or 21 of neonatal life. UCP2 protein abundance in AT was similar between genotypes on the first day of life, but it was elevated at all subsequent postnatal ages ($P < 0.05$) in AT of Meishan piglets. In contrast, UCP2 mRNA abundance was lower in Meishans up to 14 days of age. UCP2 mRNA expression was not correlated with protein abundance in either breed at any age. UCP3 mRNA in AT was similar between breeds up to day 7; thereafter, expression was higher (general linear model, $P < 0.05$) in Meishan piglets. Conversely, UCP3 mRNA expression in SM was higher in commercial piglets after day 7. Colonic temperature remained lower in Meishan than commercial piglets throughout the study; this was most obvious in the immediate post-partum period when Meishan piglets had lower ($P < 0.05$) plasma triiodothyronine. In conclusion, we have demonstrated that porcine genotype influences the expression and abundance of UCP2 and 3, an influence which may, in part, be due to the distinctive endocrine profiles associated with each genotype.

ROUSSET S., ALVES-GUERRA M.C., MOZO J., MIROUX B., CASSARD-DOULCIER A.M., BOUILLAUD F., RICQUIER D.

The biology of mitochondrial uncoupling proteins.

Diabetes, 53 Suppl 1 S130-S135, 2004

(Services cités : UPR 9078)

Uncoupling proteins (UCPs) are mitochondrial transporters present in the inner membrane of mitochondria. They are found in all mammals and in plants. They belong to the family of anion mitochondrial carriers including adenine nucleotide transporters. The term "uncoupling protein" was originally used for UCP1, which is uniquely present in mitochondria of brown adipocytes, the thermogenic cells that maintain body temperature in small rodents. In these cells, UCP1 acts as a proton carrier activated by free fatty acids and creates a shunt between complexes of the respiratory chain and ATP synthase. Activation of UCP1 enhances respiration, and the uncoupling process results in a futile cycle and dissipation of oxidation energy as heat. UCP2 is ubiquitous and highly expressed in the lymphoid system, macrophages, and pancreatic islets. UCP3 is mainly expressed in skeletal muscles. In comparison to the established uncoupling and thermogenic activities of UCP1, UCP2 and UCP3 appear to be involved in the limitation of free radical levels in cells rather than in physiological uncoupling and thermogenesis. Moreover, UCP2 is a regulator of insulin secretion and UCP3 is involved in fatty acid metabolism.

2003

ALVES-GUERRA M.C., ROUSSET S., PECQUEUR C., MALLAT Z., BLANC J., TEDGUI A., BOUILLAUD F., CASSARD-DOULCIER A.M., RICQUIER D., MIROUX B.

Bone Marrow Transplantation Reveals the in Vivo Expression of the Mitochondrial Uncoupling Protein 2 in Immune and Nonimmune Cells during Inflammation.

J. Biol. Chem., 278 (43), 42307-42312, 2003

(Services cités : UPR 9078)

The mitochondrial uncoupling protein 2 (UCP2) is expressed in spleen, lung, intestine, white adipose tissue, and immune cells. Bone marrow transplantation in mice was used to assess the contribution of immune cells to the expression of UCP2 in basal condition and during inflammation. Immune cells accounted for the total amount of UCP2 expression in the spleen, one-third of its expression in the lung, and did not participate in its expression in the intestine. LPS injection stimulated UCP2 expression in lung, spleen, and intestine in both immune and non-immune cells. Successive injections of LPS and dexamethasone or N-acetyl-cysteine prevented the induction of UCP2 in all three tissues, suggesting that oxygen free radical generation plays a role in UCP2 regulation. Finally, both previous studies and our data show that there is down-regulation of UCP2 in immune cells during their activation in the early stages of the LPS response followed by an up-regulation in UCP2 during the later stages to protect all cells against oxidative stress.

ARECHAGA I., MIROUX B., RUNSWICK M.J., WALKER J.E.

Over-expression of Escherichia coli F1F(o)-ATPase subunit a is inhibited by instability of the uncB gene transcript.

FEBS Lett., 547 (1-3), 97-100, 2003

(Services cités : UPR 9078)

Little is known about the stability of transcripts encoding membrane proteins in strong expression systems and its effect on membrane protein over-production. We have expressed all the genes encoding subunits of the membrane domain F_o of the ATP synthase in a T7 RNA polymerase-based system. All of them but uncB (subunit a) were expressed separately at very high levels in the bacterial hosts *Escherichia coli* C41(DE3) and C43(DE3). However, expression of uncB was extremely toxic to the bacteria. Northern blot analysis showed that the level of accumulation of the mRNA from uncB was very low. Deletion of uncB in combination with gene fusion experiments demonstrated that the middle region of the gene, encoding amino acids 92-171, exhibited a dominant toxic phenotype associated with a very poor level of expression. Green fluorescent protein fusions with N- and C-ends of uncB helped to stabilize the mRNA and to obtain high yields of protein.

BLANC J., ALVES-GUERRA M.C., ESPOSITO B., ROUSSET S., GOURDY P., RICQUIER D., TEDGUI A., MIROUX B., MALLAT Z.

Protective role of uncoupling protein 2 in atherosclerosis.

Circulation, 107 (3), 388-390, 2003

(Services cités : UPR 9078)

BACKGROUND: Uncoupling protein 2 (UCP2) regulates the production of reactive oxygen species in macrophages. However, its role in atherosclerosis is unknown. **METHODS AND RESULTS:** Irradiated low-density lipoprotein receptor deficient mice (LDLR^{-/-}) were transplanted with bone marrow from either UCP2 deficient mice (Ucp2^{-/-}) or wild type mice (Ucp2^{+/+}). Mice were fed an atherogenic diet for 7 weeks. Engraftment of bone marrow cells was confirmed by the presence of UCP2 protein expression in spleen cell mitochondria of Ucp2^{+/+} transplanted mice and its absence in Ucp2^{-/-} transplanted mice. Leukocyte counts and plasma cholesterol levels were comparable in both groups. We found a marked increase in atherosclerotic lesion size in the thoracic aorta of Ucp2^{-/-} transplanted mice compared with control Ucp2^{+/+} transplanted mice (8.3±0.9% versus 4.3±0.4%, respectively; P<0.005), as well as in the aortic sinus (150 066±12 388 microm² versus 105 689±9 727 microm², respectively; P<0.05). This was associated with increased nitrotyrosine staining, which suggests enhanced oxidative stress. Analysis of plaque composition revealed a significant increase in macrophage accumulation (P<0.05) and apoptosis (P<0.05), along with a decrease in collagen content (P<0.05), suggesting a potentially more vulnerable phenotype. **CONCLUSION:** These results suggest a protective role for UCP2 against atherosclerosis.

CLAVEL S., PARADIS E., RICQUIER D., RICHARD D.

Kainic acid upregulates uncoupling protein-2 mRNA expression in the mouse brain.

Neuroreport, 14 (16), 2015-2017, 2003

(Services cités : UPR 9078)

Intraperitoneal injection of kainic acid (KA) in C57BL/6J and 129T2SvEmsJ mice led to a transient induction of uncoupling protein-2 (Ucp2) mRNA expression in several brain regions, which included the CA1 subfield of the hippocampus, the dorsal endopiriform nucleus and the piriform cortex in both strains. In all those regions, levels of Ucp2 mRNA expression, as determined by in situ hybridization, peaked at 24 h and returned to basal levels within 72 h post-injection. The increase in mRNA expression was mainly observed in neurons, with microglial cells displaying only scattered expression of the gene. The neuronal induction of Ucp2 in

response to KA was stronger in 129T2SvEmsJ mice than in C57BL/6J, which suggests a role for Ucp2 in excitotoxic challenges and neuroprotection.

MOSTYN A., WILSON V., DANDREA J., YAKUBU D.P., BUDGE H., ALVES-GUERRA M.C., PECQUEUR C., MIROUX B., SYMONDS M.E., STEPHENSON T.

Ontogeny and nutritional manipulation of mitochondrial protein abundance in adipose tissue and the lungs of postnatal sheep.

Br. J. Nutr., 90 (2), 323-328, 2003

(Services cités : UPR 9078)

The present study examined the ontogeny of mitochondrial protein abundance in adipose tissue and lungs over the first month of life in the sheep and the extent to which this may be altered by maternal undernutrition during the final month of gestation. The ontogeny of uncoupling protein (UCP), voltage-dependent anion channel (VDAC) and cytochrome c abundance were determined in adipose tissue and lungs sampled from near-term fetuses and young sheep aged 4 h, 1, 7 and 30 d. In adipose tissue, the abundance of UCP1, VDAC and cytochrome c all peaked at 1 d of age and then decreased by 30 d of age, at which stage the brown adipose tissue-specific UCP1 was no longer detectable but UCP2 was clearly abundant. For the lungs, however, UCP2 and VDAC abundance both peaked 7 d after birth and then decreased by 30 d of age. During postnatal development, therefore, a marked change in mitochondrial protein abundance occurs within both adipose tissue and lungs. Maternal nutrient restriction had no effect on lamb growth or tissue weights at 30 d of age but was associated with increased abundance of UCP2 and VDAC but not cytochrome c in both adipose tissue and lungs. These mitochondrial adaptations within both adipose tissue and the lungs of offspring born to previously nutrient-restricted mothers may compromise adipose tissue and lung function during periods of environmental stress.

NACER-CHERIF H., BOIS-JOYEUX B., ROUSSEAU G.G., LEMAIGRE F.P., DANAN J.L.

Hepatocyte nuclear factor-6 stimulates transcription of the alpha-fetoprotein gene and synergizes with the retinoic-acid-receptor-related orphan receptor alpha-4.

Biochem. J., 369 (Pt 3), 583-591, 2003

(Services cités : UPR 9078)

The rat alpha-fetoprotein (*afp*) gene is controlled by three enhancers whose function depends on their interaction with liver-enriched transcription factors. The *afp* enhancer III, located at -6 kb, is composed of three regions that act in synergy. Two of these regions, called *s1* and *s2*, contain a putative binding site for hepatocyte nuclear factor-6 (HNF-6). This factor is the prototype of the ONECUT family of cut-homoeodomain proteins and is a known regulator of liver gene expression in adults and during development. We show here that the two splicing isoforms of HNF-6 bind to a site in the *s1* region and in the *s2* region. The core sequence of the *s1* site corresponds to none of the known HNF-6 binding sites. Nevertheless, the binding properties of the *s1* site are identical with those of the *s2* site and of previously characterized HNF-6 binding sequences. The HNF-6 consensus should therefore be rewritten as DRRTC VATND. Binding of HNF-6 to the *s1* and *s2* sites requires both the cut and the homoeo domains, is co-operative and induces DNA bending. HNF-6 strongly stimulates the activity of the *afp* enhancer III in transient transfection experiments. This effect requires the stereo-specific alignment of the two HNF-6 sites. Moreover, HNF-6 stimulates the enhancer in synergy with the retinoic-acid-receptor-related

orphan receptor alpha (RORalpha), which binds to a neighbouring site in the s1 region. Thus expression of the *afp* gene requires functional interactions between HNF-6 molecules and between HNF-6 and RORalpha.

PARADIS E., CLAVEL S., BOUILLAUD F., RICQUIER D., RICHARD D.

Uncoupling protein 2: a novel player in neuroprotection.

Trends Mol. Med., 9 (12), 522-552, 2003

(Services cités : [UPR 9078](#))

PEARCE S., MOSTYN A., ALVES-GUERRA M.C., PECQUEUR C., MIROUX B., WEBB R., STEPHENSON T., SYMOND M.E.

Prolactin, prolactin receptor and uncoupling proteins during fetal and neonatal development.

Proc. Nutr. Soc., 62 (2), 421-427, 2003

(Services cités : [UPR 9078](#))

Uncoupling proteins (UCP) 1 and 2 are members of the subfamily of inner mitochondrial membrane carriers. UCP1 is specific to brown adipose tissue (BAT), where it is responsible for the rapid production of heat at birth. In fetal sheep UCP1 is first detectable at approximately 90 d of gestation; its abundance increases with gestational age and peaks at the time of birth. The mRNA and protein for both the long and short form of the prolactin (PRL) receptor (PRLR) are also highly abundant in BAT. Enhanced PRLR abundance in late gestation is associated with an increase in the abundance of UCP1. This relationship between PRLR and UCP is not only present in BAT. Similar findings are now reported in the pregnant ovine uterus, where PRLR abundance reaches a maximum just before that of UCP2. However, the role of PRLR in BAT remains undetermined. Rat studies have shown that PRL administration throughout pregnancy results in offspring with increased UCP1 at birth. Studies in newborn lambs have shown that administration of PRL (2 mg/d) causes an acute response, increasing colonic temperature in the first hour by 1 degrees. This increased colonic temperature is maintained for the first 24h of life, in conjunction with enhanced lipolysis. After 7 d of treatment there is no difference in the abundance of UCP1 but an increase in UCP1 activity; this effect may be mediated by an increase in lipolysis. Taken together these findings suggest that PRL could be an important endocrine factor during pregnancy and early postnatal life.

ROUSSET S., ALVES-GUERRA M.C., OUADGHIRI-BENCHERIF S., KOZAK L.P., MIROUX B., RICHARD D., BOUILLAUD F., RICQUIER D., CASSARD-DOULCIER A.M.

Uncoupling protein 2, but not uncoupling protein 1, is expressed in the female mouse reproductive tract.

J. Biol. Chem., 278 (46), 45843-45847, 2003

(Services cités : [UPR 9078](#))

Uncoupling proteins (UCPs) are transporters of the inner mitochondrial membrane. Whereas UCP1 is uniquely present in brown adipose tissue where it uncouples respiration from ATP synthesis and activates respiration and heat production, UCP2 is present in numerous tissues, and its exact function remains to be clarified. Two sets of data provided the rationale for this study:

(i) the intriguing report that UCP1 is present in uterus of mice (Nibbelink, M., Moulin, K., Arnaud, E., Duval, C., Penicaud, L., and Casteilla, L. (2001) *J. Biol. Chem.* 276, 47291-47295); and (ii) an observation that *Ucp2*(-/-) female mice (homozygous matings) have smaller litters compared with *Ucp2*(+/+) animals (S. Rousset and A.-M. Cassard-Doulcier, unpublished observations). These data prompted us to examine the expression of UCP1 and UCP2 in the reproductive tract of female mice. Using wild type, *Ucp1*(-/-) mice, and *Ucp2*(-/-) mice, we were unable to detect UCP1 in uterus of mice with appropriate antibodies, and we conclude that the signal assigned to UCP1 by others was neither UCP1 nor UCP2. Using a polyclonal antibody against UCP2 and tissues from *Ucp2*(-/-) mice as controls, UCP2 was detected in ovary, oviduct, and uterus. Expression of *Ucp2* mRNA was also observed in ovary and uterus using in situ hybridization analysis. Bone marrow transplantation experiments revealed that the UCP2 signal of the ovary was restricted to ovarian cells. UCP2 level in ovary decreased during follicular growth and increased during the pre-ovulatory period, during which aspects of an inflammatory process are known to exist. Because UCP2 down-regulates reactive oxygen species, a role in the regulation of inflammatory events linked to the preparation of ovulation is suggested.

SHAW A.Z., MIROUX B.

A general approach for heterologous membrane protein expression in *Escherichia coli*: the uncoupling protein, UCP1, as an example.

Methods Mol. Med., 228 23-35, 2003

(Services cités : UPR 9078)

TIRABY C., TAVERNIER G., LEFORT C., LARROUY D., BOUILLAUD F., RICQUIER D., LANGIN D.

Acquirement of Brown Fat Cell Features by Human White Adipocytes.

J. Biol. Chem., 278 (35), 33370-33376, 2003

(Services cités : UPR 9078)

Obesity, i.e. an excess of white adipose tissue (WAT), predisposes to the development of type 2 diabetes and cardiovascular disease. Brown adipose tissue is present in rodents but not in adult humans. It expresses uncoupling protein 1 (UCP1) that allows dissipation of energy as heat. Peroxisome proliferator-activated receptor gamma (PPARgamma) and PPARgamma coactivator 1alpha (PGC-1alpha) activate mouse UCP1 gene transcription. We show here that human PGC-1alpha induced the activation of the human UCP1 promoter by PPARgamma. Adenovirus-mediated expression of human PGC-1alpha increased the expression of UCP1, respiratory chain proteins, and fatty acid oxidation enzymes in human subcutaneous white adipocytes. Changes in the expression of other genes were also consistent with brown adipocyte mRNA expression profile. PGC-1alpha increased the palmitate oxidation rate by fat cells. Human white adipocytes can therefore acquire typical features of brown fat cells. The PPARgamma agonist rosiglitazone potentiated the effect of PGC-1alpha on UCP1 expression and fatty acid oxidation. Hence, PGC-1alpha is able to direct human WAT PPARgamma toward a transcriptional program linked to energy dissipation. However, the response of typical white adipocyte targets to rosiglitazone treatment was not altered by PGC-1alpha. UCP1 mRNA induction was shown in vivo by injection of the PGC-1alpha adenovirus in mouse white fat. Alteration of energy balance through an increased utilization of fat in WAT may be a conceivable strategy for the treatment of obesity.

WINZELL M.S., SVENSSON H., ENERBACK S., RAVNSKJAER K., MANDRUP S., ESSER V., ARNER P., ALVES-GUERRA M.C., MIROUX B., SUNDLER F., AHREN B., HOLM C.

Pancreatic beta-cell lipotoxicity induced by overexpression of hormone-sensitive lipase.

Diabetes, 52 (8), 2057-2065, 2003

(Services cités : UPR 9078)

Lipid perturbations associated with triglyceride overstorage in beta-cells impair insulin secretion, a process termed lipotoxicity. To assess the role of hormone-sensitive lipase, which is expressed and enzymatically active in beta-cells, in the development of lipotoxicity, we generated transgenic mice overexpressing hormone-sensitive lipase specifically in beta-cells. Transgenic mice developed glucose intolerance and severely blunted glucose-stimulated insulin secretion when challenged with a high-fat diet. As expected, both lipase activity and forskolin-stimulated lipolysis was increased in transgenic compared with wild-type islets. This was reflected in significantly lower triglycerides levels in transgenic compared with wild-type islets in mice receiving the high-fat diet, whereas no difference in islet triglycerides was found between the two genotypes under low-fat diet conditions. Our results highlight the importance of mobilization of the islet triglyceride pool in the development of beta-cell lipotoxicity. We propose that hormone-sensitive lipase is involved in mediating beta-cell lipotoxicity by providing ligands for peroxisome proliferator-activated receptors and other lipid-activated transcription factors, which in turn alter the expression of critical genes. One such gene might be uncoupling protein-2, which was found to be upregulated in transgenic islets, a change that was accompanied by decreased ATP levels.

2002

ALVES-GUERRA M.C., PECQUEUR C., SHAW A., COUPLAN E., GONZALEZ-BARROSO-MADRID M.D.M., RICQUIER D., BOUILLAUD F., MIROUX B.

The uncoupling proteins family : from thermogenesis to the regulation of ROS.

in: *Sensing, Signalling, and Cell Adaptation*. (Storey K.B., Storey J.M. eds.)

Elsevier Science B.V. (), 2002, pp.257-268.

(Services cités : UPR 9078)

CHAUVET C., BOIS-JOYEUX B., DANAN J.L.

Retinoic acid receptor-related orphan receptor (ROR) alpha4 is the predominant isoform of the nuclear receptor RORalpha in the liver and is up-regulated by hypoxia in HepG2 human hepatoma cells.

Biochem. J., 364 (Pt 2), 449-456, 2002

(Services cités : UPR 9078)

The retinoic acid receptor-related orphan receptor alpha (RORalpha) is critically involved in many physiological functions in several organs. We find that the main RORalpha isoform in the mouse liver is the RORalpha4 isoform, in terms of both mRNA and protein levels, while the RORalpha1 isoform is less abundant. Because hypoxia is a major feature of liver physiology and pathology, we examined the effect of this stress on Rora gene expression and RORalpha

transcriptional activity. HepG2 human hepatoma cells were cultured for 24 h under normoxia (20% O₂) or hypoxia (10, 2, and 0.1% O₂) and the abundance of the Rora transcripts measured by Northern blot and semi-quantitative RT-PCR. Hypoxic HepG2 cells contained more Rora mRNA than controls. This was also observed in rat hepatocytes in primary culture. Cobalt chloride and desferrioxamine also increased the amount of Rora mRNA in HepG2 cells. It is likely that these treatments increase the amount of the RORalpha4 protein in HepG2 cells as evidenced by Western blotting in the case of desferrioxamine. Transient transfection experiments indicated that hypoxia, cobalt chloride, and desferrioxamine all stimulate RORalpha transcriptional activity in HepG2 cells. Hence, we believe that RORalpha participates in the control of gene transcription in hepatic cells and modulates gene expression in response to hypoxic stress.

CINTI S., CANCELLO R., ZINGARETTI M.C., CERESI E., de MATTEIS R., GIORDANO A., HIMMS-HAGEN J., RICQUIER D.

CL316,243 and cold stress induce heterogeneous expression of UCP1 mRNA and protein in rodent brown adipocytes.

J. Histochem. Cytochem., 50 (1), 21-31, 2002

(Services cités : [UPR 9078](#))

Uncoupling protein 1 (UCP1), the mammalian thermogenic mitochondrial protein, is found only in brown adipocytes, but its expression by immunohistochemistry is not homogeneous. Here we present evidence that the non-homogeneous pattern of immunostaining for UCP1 (referred to as the "Harlequin phenomenon") is particularly evident after acute and chronic cold (4C) stimulus and after administration of a specific beta(3)-adrenoceptor agonist (CL316,243). Accordingly, mRNA in situ expression confirmed the UCP1 non-homogeneous pattern of gene activation under conditions of adrenergic stimulus. Furthermore, morphometric analysis of immunogold-stained thin sections showed that UCP1-gold particle density was different among neighboring brown adipocytes with mitochondria of the same size and density. When the adrenergic stimulus was reduced in warm-acclimated animals (28C), UCP1 protein and mRNA expression was reduced and consequently the Harlequin phenomenon was barely visible. These data suggest the existence of an alternative and controlled functional recruitment of brown adipocytes in acute adrenergically stressed animals, possibly to avoid heat and metabolic damage in thermogenically active cells. Of note, the heat shock protein heme oxygenase 1 (HO1) is heterogeneously expressed in adrenergically stimulated brown adipose tissue and, specifically, cells expressing strong immunoreactivity for UCP1 also strongly express HO1.

COUPLAN E., GELLY C., GOUBERN M., FLEURY C., QUESSON B., SILBERBERG M., THIAUDIERE E., MATEO P., LONCHAMPT M., LEVENS N., de MONTRION C., ORTMANN S., KLAUS S., GONZALEZ-BARROSO-MADRID M.D.M., CASSARD-DOULCIER A.M., RICQUIER D., BIGARD A.X., DIOLEZ P., BOUILLAUD F.

High Level of Uncoupling Protein 1 Expression in Muscle of Transgenic Mice Selectively Affects Muscles at Rest and Decreases Their Iib Fiber Content.

J. Biol. Chem., 277 (45), 43079-43088, 2002

(Services cités : [UPR 9078](#))

The mitochondrial uncoupling protein of brown adipose tissue (UCP1) was expressed in skeletal muscle and heart of transgenic mice at levels comparable with the amount found in brown

adipose tissue mitochondria. These transgenic mice have a lower body weight, and when related to body weight, food intake and energy expenditure are increased. A specific reduction of muscle mass was observed but varied according to the contractile activity of muscles. Heart and soleus muscle are unaffected, indicating that muscles undergoing regular contractions, and therefore with a continuous mitochondrial ATP production, are protected. In contrast, the gastrocnemius and plantaris muscles showed a severely reduced mass and a fast to slow shift in fiber types promoting mainly IIa and IIx fibers at the expense of fastest and glycolytic type IIb fibers. These observations are interpreted as a consequence of the strong potential dependence of the UCP1 protonophoric activity, which ensures a negligible proton leak at the membrane potential observed when mitochondrial ATP production is intense. Therefore UCP1 is not deleterious for an intense mitochondrial ATP production and this explains the tolerance of the heart to a high expression level of UCP1. In muscles at rest, where ATP production is low, the rise in membrane potential enhances UCP1 activity. The proton return through UCP1 mimics the effect of a sustained ATP production, permanently lowering mitochondrial membrane potential. This very likely constitutes the origin of the signal leading to the transition in fiber types at rest.

COUPLAN E., GONZALEZ-BARROSO-MADRID M.D.M., ALVES-GUERRA M.C., RICQUIER D., GOUBERN M., BOUILLAUD F.

No evidence for a basal, retinoic, or superoxide-induced uncoupling activity of the uncoupling protein 2 present in spleen or lung mitochondria.

J. Biol. Chem., 277 (29), 26268-26275, 2002

(Services cités : UPR 9078)

The phenotypes observed in mice whose uncoupling protein (Ucp2) gene had been invalidated by homologous recombination (Ucp2^{-/-}) mice) are consistent with an increase in mitochondrial membrane potential in macrophages and pancreatic beta cells. This could support an uncoupling (proton transport) activity of UCP2 in the inner mitochondrial membrane in vivo. We used mitochondria from lung or spleen, the two organs expressing the highest level of UCP2, to compare the proton leak of the mitochondrial inner membrane of wild-type and Ucp2^{-/-} mice. No difference was observed under basal conditions. Previous reports have concluded that retinoic acid and superoxide activate proton transport by UCP2. Spleen mitochondria showed a higher sensitivity to retinoic acid than liver mitochondria, but this was not caused by UCP2. In contrast with a previous report, superoxide failed to increase the proton leak rate in kidney mitochondria, where no UCP2 expression was detected, and also in spleen mitochondria, which does not support stimulation of UCP2 uncoupling activity by superoxide. Finally, no increase in the ATP/ADP ratio was observed in spleen or lung of Ucp2^{-/-} mice. Therefore, no evidence could be gathered for the uncoupling activity of the UCP2 present in spleen or lung mitochondria. Although this may be explained by difficulties with isolated mitochondria, it may also indicate that UCP2 has another physiological significance in spleen and lung.

HOURTON-CABASSA C., MESNEAU A., MIROUX B., ROUSSAUX J., RICQUIER D., ZACHOWSKI A., MOREAU F.

Alteration of Plant Mitochondrial Proton Conductance by Free Fatty Acids. UNCOUPLING PROTEIN INVOLVEMENT.

J. Biol. Chem., 277 (44), 41533-41538, 2002

(Services cités : UPR 9078)

We characterized the uncoupling activity of the plant uncoupling protein from *Solanum tuberosum* (StUCP) using mitochondria from intact potato tubers or from yeast (*Saccharomyces cerevisiae*) expressing the StUCP gene. Compared with mitochondria from transfected yeast, StUCP is present at very low levels in intact potato mitochondrial membranes (at least thirty times lower) as shown by immunodetection with anti-UCP1 antibodies. Under conditions that ruled out undesirable effects of nucleotides and free fatty acids on uncoupling activity measurement in plant mitochondria, the linoleic acid-induced depolarization in potato mitochondria was insensitive to the nucleotides ATP, GTP, or GDP. In addition, sensitivity to linoleic acid was similar in potato and in control yeast mitochondria, suggesting that uncoupling occurring in potato mitochondria was because of a UCP-independent proton diffusion process. By contrast, yeast mitochondria expressing StUCP exhibited a higher sensitivity to free fatty acids than those from the control yeast and especially a marked proton conductance in the presence of low amounts of linoleic acid. However, this fatty acid-induced uncoupling was also insensitive to nucleotides. Altogether, these results suggest that uncoupling of oxidative phosphorylation and heat production cannot be the dominant feature of StUCP expressed in native potato tissues. However, it could play a role in preventing reactive oxygen species production as proposed for mammalian UCP2 and UCP3.

LAFUSTE P., ROBERT B., MONDON F., DANAN J., ROSSI B., DUC-GOIRAN P., MIGNOT T., NUNEZ E., BENASSAYAG C., FERRE F.

Alpha-fetoprotein Gene Expression in Early and Full-term Human Trophoblast.

Placenta, 23 (8-9), 600-612, 2002

(Services cités : [UPR 9078](#))

Alpha-fetoprotein (AFP) is a major serum glycoprotein synthesized during fetal life mainly by the yolk sac and the fetal liver. At term, it reaches high concentrations in the maternal intervillous blood, which is in direct contact with the placental trophoblastic microvillous membrane, and this suggests the placental origin of the AFP at the fetal-maternal interface. We used several experimental approaches to investigate the expression of AFP gene and fetal protein production in early gestation and term placentas. RT-PCR and immunological studies clearly identified AFP messenger RNA and AFP protein in the placental villi from first trimester of pregnancy. The AFP gene was also expressed in highly purified cytotrophoblasts from early placentas, and enzyme-immunoassay showed that AFP protein was synthesized and secreted by early cytotrophoblasts. AFP was also detected in the cytoplasm of these cells by immuno-cytochemistry. However, none of these methods detected any expression of the AFP gene in full-term placental villi or in cultured trophoblasts. These findings demonstrate that both AFP mRNA and protein are present in trophoblastic cells early in pregnancy. The absence of AFP gene expression in term placental villi also suggests, that the AFP at the fetal-maternal interface is attributable to a notable transplacental passage of AFP from fetal blood in late pregnancy.

MAZURE N.M., CHAUVET C., BOIS-JOYEUX B., BERNARD M.A., NACER-CHERIF H., DANAN J.L.

Repression of alpha-fetoprotein gene expression under hypoxic conditions in human hepatoma cells: characterization of a negative hypoxia response element that mediates opposite effects of hypoxia inducible factor-1 and c-Myc.

Cancer Res., 62 (4), 1158-1165, 2002

(Services cités : [UPR 9078](#))

Hypoxia is an important component of many pathological processes including cancerogenesis and cirrhosis. We have attempted to identify additional hepatic genes sensitive to hypoxia by postulating that genes with possible binding sites for hypoxia inducible factor-1 (HIF-1) are regulated by hypoxia. A computer analysis identified the oncodevelopmental alpha-fetoprotein gene (afp) as one of them. The amounts of both alpha-fetoprotein mRNA and protein were decreased under hypoxic conditions in HepG2 hepatoma cells. Stability of afp mRNA was not altered, and de novo synthesis of proteins was required. Transfection experiments in HepG2 cells showed that both hypoxia and overproduction of HIF-1alpha specifically repressed the transcriptional activity of the rat afp regulatory region through the sequence 5'-CACGTGGG-3' located at -3625 to -3619. Mutation in this sequence strongly impaired these repressions. Interestingly, this sequence was a functional stimulatory target for c-Myc, suggesting that c-Myc regulates afp gene expression. Lastly, the amounts of c-myc mRNA and protein were reduced when these cells were grown under hypoxic conditions. Taken together, these results suggest the existence of a possible competition between HIF-1 and c-Myc that could modulate the transcriptional activity of the afp gene in response to hypoxia.

NICOLAS G., CHAUVET C., VIATTE L., DANAN J.L., BIGARD X., DEVAUX I., BEAUMONT C., KAHN A., VAULONT S.

The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation.

J. Clin. Invest., 110 (7), 1037-1044, 2002

(Services cités : [UPR 9078](#))

The present study was aimed at determining whether hepcidin, a recently identified peptide involved in iron metabolism, plays a role in conditions associated with both iron overload and iron deficiency. Hepcidin mRNA levels were assessed in two models of anemia, acute hemolysis provoked by phenylhydrazine and bleeding provoked by repeated phlebotomies. Hepcidin response to hypoxia was also studied, both ex vivo, in human hepatoma cells, and in vivo. Anemia and hypoxia were associated with a dramatic decrease in liver hepcidin gene expression, which may account for the increase in iron release from reticuloendothelial cells and increase in iron absorption frequently observed in these situations. A single injection of turpentine for 16 hours induced a sixfold increase in liver hepcidin mRNA levels and a twofold decrease in serum iron. The hyposideremic effect of turpentine was completely blunted in hepcidin-deficient mice, revealing hepcidin participation in anemia of inflammatory states. These modifications of hepcidin gene expression further suggest a key role for hepcidin in iron homeostasis under various pathophysiological conditions, which may support the pharmaceutical use of hepcidin agonists and antagonists in various iron homeostasis disorders.

RICQUIER D.

To burn or to store.

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(Services cités : [UPR 9078](#))

Energy exists as organic molecules and heat in living organisms. In adult mammals, body weight and fat content remain unchanged if energy intake is strictly equivalent to energy expenditure. In other words, regulation of body weight requires energy of foods to be entirely dissipated as heat.

imbalance between ingested energy and thermogenesis induces obesity or thinness. Alterations of food intake or energy expenditure represent the two causes of body weight disturbance. It is accepted that individuals differ in food efficiency i.e. ability to metabolize foods and store fat or totally burn nutrients. Mechanisms of food efficiency and futile cycles are presented. I started my research work analysing thermogenic mechanism in brown adipose tissue. Actually, in addition to white adipose tissue which is the major type of adipose tissue, mammals own another type of adipose tissue referred to as brown adipose tissue. This latter tissue is an activatable thermogenic organ which oxidises fatty acids and releases heat in blood stream. Brown fat is activated during exposure to the cold (in rodents), at birth, and during arousal in hibernators. My initial work helped to characterize a mitochondrial protein named uncoupling protein or UCP which is responsible for activation of fatty acid oxidation and heat production in brown adipocytes. Actually, in most cells, fifty per cent of oxidation energy is recovered as ATP in mitochondria through the process of coupling of respiration to ADP phosphorylation. In contrast to mitochondria of most tissues, brown adipocyte mitochondria can escape the obligatorily coupling of respiration and waste almost ninety per cent of respiration energy as thermogenesis. UCP characterization and its molecular cloning as well as antibodies obtention were used to better understand cellular thermogenesis. Brown adipocytes were identified in babies and adult patients with pheochromocytoma. More recently, research on the brown fat UCP helped us to identify UCP2, a UCP homolog present in most human and animal tissues. A family of UCPs exist in animals and plants. These UCPs may function as mitochondrial uncouplers. However, the ancient function of the UCPs may be rather associated to adaptation to oxygen and control of free radicals than to thermogenesis. Further studies of UCPs will improve the knowledge of mitochondrial metabolism and substrate oxidation. In other respects, analysis of molecular mechanisms controlling respiration uncoupling may contribute to new strategies of treatment of metabolic disorders such as obesity.

ROUSSET S., GONZALEZ-BARROSO-MADRID M.D.M., GELLY C., PECQUEUR C., BOUILLAUD F., RICQUIER D., CASSARD-DOULCIER A.M.

A new polymorphic site located in the human UCP1 gene controls the in vitro binding of CREB-like factor.

Int. J. Obes. Relat. Metab. Disord., 26 (5), 735-738, 2002

(Services cités : UPR 9078)

Uncoupling protein 1 (UCP1) is uniquely expressed in brown adipose tissue (BAT) and generates heat by uncoupling respiration from ATP synthesis. A defect in BAT thermogenesis has been described in different models of rodent obesity. In humans, the implication of BAT in energy expenditure is still under discussion. A BclI polymorphism associated with fat gain over time has been described in the upstream region of the human UCP1 (hUCP1) gene. In this study, a new polymorphic site linked to the BclI site is described which results in a C to A point mutation, 89 bp downstream of the BclI site, i.e. at position -3737 bp. This site is located in the recently analysed regulatory region of the hUCP1 gene. The mutation disrupts a consensus site for the binding of ATF/CREB transcription factor family and inhibits the factor binding in vitro.

However, transient transfection of a rodent brown adipocyte cell line shows that the isoproterenol (ISO) stimulation of the hUCP1 gene transcription is not significantly affected by this mutation.

However, we postulate that the C/A substitution, in human, may contribute to a minor defect in the regulation of hUCP1 transcription and that would explain fat gain over time.

VETTOR R., FABRIS R., SERRA R., LOMBARDI A.M., TONELLO C., GRANZOTTO M., MARZOLO M.O., CARRUBA M.O., RICQUIER D., FEDERSPIL G., NISOLI E.

Changes in FAT/CD36, UCP2, UCP3 and GLUT4 gene expression during lipid infusion in rat skeletal and heart muscle.

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OBJECTIVE: It has been reported that an increased availability of free fatty acids (NEFA) not only interferes with glucose utilization in insulin-dependent tissues, but may also result in an uncoupling effect of heart metabolism. We aimed therefore to investigate the effect of an increased availability of NEFA on gene expression of proteins involved in transmembrane fatty acid (FAT/CD36) and glucose (GLUT4) transport and of the uncoupling proteins UCP2 and 3 at the heart and skeletal muscle level. **STUDY DESIGN:** Euglycemic hyperinsulinemic clamp was performed after 24 h Intralipid(R) plus heparin or saline infusion in lean Zucker rats. Skeletal and heart muscle glucose utilization was calculated by 2-deoxy-[1-(3)H]-D-glucose technique. Quantification of FAT/CD36, GLUT4, UCP2 and UCP3 mRNAs was obtained by Northern blot analysis or RT-PCR. **RESULTS:** In Intralipid(R) plus heparin infused animals a significant decrease in insulin-mediated glucose uptake was observed both in the heart (22.62±2.04 vs 10.37±2.33 ng/mg/min; P<0.01) and in soleus muscle (13.46±1.53 vs 6.84±2.58 ng/mg/min; P<0.05). FAT/CD36 mRNA was significantly increased in skeletal muscle tissue (+117.4±16.3%, P<0.05), while no differences were found at the heart level in respect to saline infused rats. A clear decrease of GLUT4 mRNA was observed in both tissues. The 24 h infusion of fat emulsion resulted in a clear enhancement of UCP2 and UCP3 mRNA levels in the heart (99.5±15.3 and 80±4%) and in the skeletal muscle (291.5±24.7 and 146.9±12.7%). **CONCLUSIONS:** As a result of the increased availability of NEFA, FAT/CD36 gene expression increases in skeletal muscle, but not at the heart level. The augmented lipid fuel supply is responsible for the depression of insulin-mediated glucose transport and for the increase of UCP2 and 3 gene expression in both skeletal and heart muscle.