# Involvement of Cx43 in the hypothalamic glucose-sensing

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# Abstracts

### Circadian clocks and feeding behavior

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Biological functions display daily rhythms, including feeding/lipogenesis during the active period and fasting/lipolysis during the resting period. Such a temporal organization is controlled by a circadian timing system made of interconnected endogenous clocks and oscillators. The master circadian clock, located in the suprachiasmatic nuclei of the hypothalamus, is mainly reset by light and synchronizes peripheral oscillations. The secondary clocks/oscillators, present in many brain regions and peripheral organs (e.g. liver and white adipose tissue), can be shifted by meal timing, as modulated by temporal restricted feeding, while the suprachiasmatic clock is relatively impervious to the impact of meal time. However, timed calorie restriction (i.e. when only a hypocaloric diet is given every day) is able to modify the suprachiasmatic clockwork and to modulate synchronisation to light, via increased phase-shifting effects of light. High-fat feeding also affects the suprachiasmatic clockwork and modulates synchronisation to light, via reduced shifting effects of light.

Secondary clocks in the brain outside the suprachiasmatic nuclei are differentially influenced by meal timing. Circadian oscillations can be either highly sensitive to feeding cues (i.e. their phase is shifted according to meal schedule) in some structures (e.g. paraventricular hypothalamic nuclei and cerebellum) or hardly affected in others (e.g. hippocampus). Furthermore, the circadian anticipation of meal time relies on cerebellum integrity.

These data indicate that feeding cues can markedly modulate the timing of the circadian system, not only at the periphery, but also within the brain. The light-entrainable clock in the suprachiasmatic nuclei, which drives the sleep-wake cycle, is only sensitive to nutritional cues associated with metabolically challenging conditions. The cerebral clocks sensitive to meal time, such as those in the metabolic hypothalamus and cerebellum, define a network of coupled meal-entrainable oscillators controlling the feeding cycle.

Numerous metabolic processes, like adipogenesis, are regulated by transcriptional networks shared by circadian clocks. Obesity and diabetes are associated with circadian alterations. Conversely, circadian dysfunctions, either due to impaired clockwork (e.g. knock-out of clock genes) or impaired synchronisation (e.g. chronic jet-lag or shift-work), are associated with increased metabolic risks. A chronic desynchronisation can trigger fat overload, leading to a so-called 'chronobesity'.

Supported by: CNRS, University of Strasbourg, ANR JCJC

### Morbidly obese patients and drug: the clinical pharmacologist's view

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Drug lipophilicity, organ blood flow , tissue binding, drug plasma protein binding and the ionization state are the ABCs of pharmacokineticists. Most of us will consider that a lipophilic drug administered to an obese patient will lead to an increase of the peripheral distribution and perhaps to a tissue accumulation. However, we have to admit that few studies are performed in obese patients and even more rarely on morbidly obese. Meanwhile we all teach these concepts hoping that they are generally true. Science is hard and unsympathetic with us and reading the results of studies should encourage us to be more circumspect. Indeed, even if lipophilicity could be an important determinant for some drugs, others are less well predicted by this physicochemical property. Obesity is linked to a number of co-morbidities (i.e. heart insufficiency, hypertension) or to modification of the way of life (decrease in exercise), which could also affect pharmacokinetic parameters. The volume of distribution changes in the obese as well as clearance is drug-specific. So, should we have to recommend performing pharmacokinetic studies in obese patients for all drug development? This question has to be discussed as well as which design should be used, full profile and non-compartmental analysis or population pharmacokinetic modeling? Furthermore, which metric should be test, body weight, body mass index, body surface area…? All these issues will be addressed but all should not be definitively settled.

### Is obesity a disease or only an adaptation?

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Obesity is increasing all around the world at an epidemic rate. Although France is not the most affected country, the last OBEPI study shows that 14.5% of the population was obese and 31.9% overweight. In fact, only 50% of the population has a normal body weight.

Increasing body weight is an adaptation to the large changes in life-style that are occurring with a decrease in physical activity and the development of sedentary behaviours as well as changes in food habits patterns. This leads to a positive energy balance and the body has no other solution than to increase fat mass. The increase in obesity prevalence is problematic, as this condition is associated with health complications such as diabetes and cardiovascular diseases, more particularly when the excess body fat is stored in the deep abdominal region. Cut-off values for obesity, de?ned as a body mass index (BMI, kg = $m^2$)>30 come from the epidemiological observations of the large increase in mortality risk above this level. However, we can found some people with large obesity and lacking of metabolic alteration usually associated to obesity (obese metabolically normal), subjects only prone to mechanical complications of obesity. On the other hand, patients with total lack of fat, as seen in lipoatrophic diabetes, have also important metabolic complications. Understanding the underlying mechanisms leading to ectopic fat accumulation are of major importance to detect subjects for whom weight gain will be particularly deleterious. However on a Public Health perspective, labelling obesity as a disease is a necessary step in a campaign to combat obesity. Prevention by deep changes in lifestyle is mandatory as many of chronic diseases such as diabetes, cardio-vascular diseases, cancer could be prevented by better management of body weight.

### Mechanisms and consequences of human adipose tissue inflammation

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Obesity, defined as an excess of white adipose tissue mass, is considered a sterile and chronic inflammatory state, characterized by increased circulating levels of inflammatory factors as cytokines and chemokines. It is now widely recognized that the adipose tissue it-self is a site of inflammation in obesity. In this talk, I will provide examples to show how transcriptomic analysis increased our knowledge of obesity-linked adipose tissue pathology. Our initial studies detected major alterations in inflammation-related genes, including increased expression of macrophage markers, in obese adipose tissue. Macrophage accumulation was confirmed by immunohistochemistry, with a more marked effect of obesity in visceral than subcutaneous fat depot. A positive relationship between the number of macrophages in visceral fat and the severity of non-alcoholic hepatic histopathology was found in a large population of obese subjects (Tordjman et al, *J Hepatol*, 2009; 51: 354–62). This suggests that macrophage accumulation contributes to the well-known association between abdominal obesity and metabolic complications. The mechanisms relaying immune cell infiltration in hypertrophied adipose tissue are the focus of intense research. We contributed to show the importance of chemokines, including CCL5/RANTES, which promote monocyte diapedesis and macrophage survival (Keophiphath et al, *Arterioscler Thromb Vasc Biol*, 2010; 30: 39–45). Further evaluation of transcriptomic interactions characterizing human adipose tissue showed a strong relationship linking inflammation to extracellular matrix components. Indeed, the obese adipose tissue displays large fibrotic areas, with distinct pattern and composition according to tissue anatomic location (Divoux et al, *Diabetes*, 2010; 59: 2817–25). Various cell types, including macrophages, mast cells and pre-adipocytes were detected in fibrotic bundles. Isolated human pre-adipocytes acquire a pro-fibrotic phenotype when cultured with activated macrophage conditioned media (Keophiphath et al, *Mol Endocrinol*, 2009; 23:11–24) suggesting their contribution to fibrosis deposition in the inflammatory microenvironment of obese adipose tissue. Thus, the inflamed adipose tissue undergoes complex and interrelated alterations in obesity. The challenging task of determining their causes and consequences is a prerequisite for new therapeutic approaches targeting adipose tissue homeostasis.

### Obesity and cancer: therapeutics aspects

**S Hamza**, B Guiu, P Hillon

The knowledge of the mechanisms involved in the relationship between obesity and cancer may help us to better understand the therapeutic aspects in cancer prevention and treatment in overweight patients. The mechanisms involved in carcinogenesis related to obesity are for a large part in connection with an excess of visceral fat. Visceral fat is responsible not only for an increased risk of cancer but also for a worsened severity and in some cases for loss of drug efficacy. These deleterious effects are essentially due to dysfunctional visceral adipose tissue. Changes in the physiological functions of adipose tissue lead to insulin resistance, chronic inflammation and altered secretion of adipokines.

Insulin resistance due to inflammation and free fatty acid excess contributes to the increased risk of cancer in obese people. Insulin resistance induces insulin secretion by pancreatic beta-cells and increases bio-availability of IGF-1, responsible for cellular growth and decreased apoptosis. The role of Insulin resistance explains the protective effect of drugs increasing insulin sensitivity like metformin, against hepatocellular carcinoma risk.

Altered secretion of adipokines in obese patients results from pro-inflammatory cytokines, leptin, VEGF and plasminogen activator inhibitor-1 (PAI-1) hypersecretion, and decreased adiponectin secretion. Adipokin abnormalities are involved in different carcinogenesis steps: cellular proliferation and apoptosis, angiogenesis, and extracellular alterations through the activation of matrix metalloproteinases leading to tumour growth, progression, and metastasis. Some of these mechanisms will be potential therapeutic targets in Human cancer in the future. They should explain the decreased efficacy of anti-angiogenic drugs described in high visceral fat volume patients suffering from metastatic colorectal and renal cancer.

The fight against visceral fat excess is based on nutritional recommendations and physical activity. In the future, some new antiangiogenic agents specifically inhibiting angiogenic receptors of visceral adipose tissue, could take a predominant place in obesity treatment. These drugs that are effective on weight loss in obese animals, are currently evaluated in patients suffering from metastatic prostate cancer whose severity seems to be strongly related to visceral obesity.

**Conclusion:** The expression of *ATG12* in blood cells of SCA7 patients is correlated with disease severity. Other genes involved in the autophagic pathway are also deregulated in accordance with previous study showing elevated autophagy markers in mouse cerebellum. Although to be confirmed in independent studies, our results suggest new therapeutic strategies in this rare disease and provide a potential biomarker to follow disease course and treatment effects.

### 26-O054

**TRPV1 in brain is involved in paracetamol-induced antinociception**

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**Background:** In 1948, Brodie and Axelrod demonstrated that paracetamol is the major active metabolite of acetanilide in man. Since then, paracetamol has become one of the most popular over-the-counter analgesic and antipyretic agents, consumed by millions of people daily. However, its mechanism of action is still a matter of debate. We have previously shown that paracetamol is further metabolized to *N*-(4-hydroxyphenyl)-(5Z,8Z,11Z,14Z)-icosatetra-5,8,11,14-enamide (AM404) by fatty acid amide hydrolase (FAAH) in the brain and that this metabolite is a potent activator of transient receptor potential vanilloid 1 ($TRPV_1$) in vitro. Pharmacological activation of $TRPV_1$ in the midbrain periaqueductal gray elicits antinociception in rats.

**Aim:** It is therefore possible that activation of $TRPV_1$ in brain contributes to the analgesic effect of paracetamol.

**Materials and methods:** Thermal, mechanical and chemical pain tests in rat and mice were used to assessed the analgesic effet of drugs ($n$ = 6–8 animals *per* group). Pharmacological and genetical (knock-out mice) approches were used to study the involvement of $TRPV_1$ receptor. Prostanoids and endocannabinoids were assessed by a liquid chromatography system with an autosampler coupled to a tandem mass spectrometer (LC-MS-MS).

**Results:** Here we show that the antinociceptive effect of paracetamol at an oral dose lacking hypolocomotor activity, is absent in FAAH and $TRPV_1$ knockout mice in different pain tests. This dose of paracetamol did not affect the global brain contents of prostaglandin $E_2$ ($PGE_2$) and endocannabinoids. Intracerebroventricular injection of AM404 produced a $TRPV_1$-mediated antinociceptive effect in the mouse formalin test. Pharmacological inhibition of $TRPV_1$ in the brain by intracerebroventricular capsazepine injection abolished the antinociceptive effect of oral paracetamol in the same test.

**Discussion:** This study shows that TRPV1 in brain is involved in the antinociceptive action of paracetamol and provides a strategy for developing central nervous system active oral analgesics based on the coexpression of FAAH and TRPV1 in the brain.

### 26-P119

**Human Merkel cell in culture express thermosensitive ion channels**

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Skin is the body organ of touch and thermo-sensation. Slowly adapting type I receptors in vertebrate are identified with Merkel cells connected to Ab nerve endings. It has been suggested that Merkel cells are mechano- and thermotransducers that signal to adjacent nerve. Nevertheless, the molecular identity of the Merkel cell ion channels and the functioning of these complexes remain poorly understood. Our objective was to develop a method for culturing Human Merkel cells to characterize ion channels sensitive to thermal stimuli. We used the expression of surface protein to sort on affinity column Merkel cells from other cells of the epidermis. Cell extract contained 80% of Merkel cells immuno-positive for cytokeratine 20, 18 and 8. Using patch-clamp and calcium imaging, we determined the response of Merkel cells to mechanical and thermal stimulation. Merkel cell swelling, hot and cold temperature provoked calcium entry. TRPM8 and TRPV3 mRNAs were identified by PCR and channel activities were induced by the agonist menthol, eucalyptol and 2-APB. These results demonstrate that human Merkel cells express thermo-TRP channels that may act as transducer of skin sensation.

### 26-P120

**Functional rehabilitation of social communication in young children with autism: clinical and neurobiological correlates**

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**Background:** The exchange and development therapy (EDT) is applied in very young children with autism. The EDT consists in the early and harmonious re-education of the basic neurophysiologic functions involved in communication abilities. Previous studies have confirmed the efficiency of the EDT in autism but the cerebral correlates that underlie these improvements have never been studied.

**Objectives:** The aim of the current study was to investigate the evolution of clinical and neurophysiological markers (ocular exploration, electrophysiological responses to face) before and after the EDT.

**Methods:** Twenty-nine children, aged 2–8 years, with severe autism and moderate to severe mental retardation were recruited. Changes in autistic symptomatology were evaluated with the BSE scale (Behavioural Summarized Evaluation scalerevised) at the beginning of treatment and 10 months later. Neurophysiological evaluations were assessed for six of these children: before the beginning T0, 1 year after T1 and 2 years after T2. Using an eye-tracking method, the visual exploration of face was investigated and visual ERPs (P1, N170) were recorded during an implicit emotional task. A group of typically developing children (TD) matched by chronological age also participated to the eye-tracking and ERPs recordings.

**Results:** ASDs displayed strongly abnormal pattern of face exploration (children with ASDs looked less at the eye region compared to TD children) and visual ERPs in response to face (P1 and N170 were delayed and smaller in ASD) confirming that these processes, involved in social adaptation, were affected in our sample at T0. One year after the beginning of the therapy, improvements were observed in both socio emotional and cognitive area. During the firts year the time spent on the eyes increased. Electrophysiological indices appeared to be affected by the EDT only during the second year. The amplitude of P1 and N170 increased and the topographical analysis revealed a normalization of the N170.

**Conclusion:** Both clinical and neurophysiological markers appeared to be affected by the EDT. Although these data are only preliminary, they are very encouraging and suggest an effect of therapy on brain development in agreement with the principles of the EDT.

### 26-P121

**Involvement of Cx43 in the hypothalamic glucose-sensing**

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**Introduction:** The hypothalamus is implicated in the nervous regulation of the glucose homeostasis. Detection of increased blood glucose level by specific hypothalamic glucose sensitive neurons triggers physiologic responses such as increased insulin secretion and decreased food intake. Astrocytes are suspected to be involved in the brain glucose-sensing. They present a network organization formed by numerous connexin gap-junctions (GJ). This allows the transfer of glucose from bloodstream to neurons. The major connexin expressed in astrocytes is the connexin 43 (Cx43). We hypothesized that the Cx43-dependent astrocyte networks plays a critical role in hypothalamic glucose-sensing.

**Materials and methods:** Expression of Cx43 was studied by immunochemistry in rat ventro-median hypothalamus (VMH). Decrease in VMH Cx43 expression was assessed, in vivo, by stereotaxic injection of siRNA against Cx43. Evaluation of hypothalamic glucose-sensing was assessed by monitoring insulin secretion or refeeding of 20 h-fasted animals in response to an intracarotid injection of a glucose bolus towards the brain.

**Results:** Cx43 is highly expressed in the VMH at the astrocytic end-feet, around blood vessels. Inhibition of Cx43 (about 30%) leads, 72 h after the injection, to a decrease of food intake whitout modification of weigth, blood glucose or insulin levels. Intracarotid glucose injection-induced insulin secretion is significantly decreased in siCx43 rats. Similarly, the satietogenic effect of glucose is significantly attenuated in 20 h-fasted siCx43 rats during the refeeding.

**Conclusion:** These results show that Cx43-dependant astrocyte networks are critical for hypothalamic glucose sensitivity.

### 26-P122

**The neuroretina is a novel mineralocorticoid target organ: aldosterone up-regulates ion and water channels in Müller glial cells**

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Retinal Müller glial cells (RMG) are key elements for the control of retina hydration and homeostasis of potassium, as they establish an anatomical and functional connection between the retinal neurons and the retinal blood vessels on one hand, and with the vitreous and the subretinal space on the other hand. Any alteration in the retinal dehydration processes leads to retinal edema. Glucocorticoids (G) reduce diabetic macular edema, but the mechanisms underlying G effects are imperfectly elucidated. G may bind to glucocorticoid (GR) and also mineralocorticoid (MR) receptors. We hypothesize that MR activation may influence retinal hydration. The effect of the MR agonist aldosterone (24 h) on ion/water channels expression (real-time PCR, western blot, immunofluorescence) was investigated on cultured retinal Müller glial cells (RMG, that contribute to fluid homeostasis in the retina), in Lewis rat retinal explants and in rat retinas following intra-vitreous aldosterone injection. We evidenced cell-specific expression of MR, GR and 11-beta hydroxysteroid dehydrogenase type II. Aldosterone significantly enhances expression of sodium and potassium channels ENaC- $\alpha$ (6.5-fold) and Kir4.1 (1.9-fold) through MR and GR occupancy, while aquaporin 4 (AQP4, 2.9-fold up-regulation) is MR-selective. Aldosterone intravitreous injection induces retinal swelling (24% increase compared to sham-injected eyes) and activation of RMG. It promotes additional localization of Kir4.1 and AQP4 towards apical microvilli of RMG. Our results highlight the mineralocorticoid-sensitivity of the neuroretina and show that aldosterone controls hydration of the healthy retina through regulation of ion/water channels expression in RMG. These results provide a rationale for future investigations of abnormal MR signalling in the pathological retina.

### 26-P123

**Evaluation of an educational Program in Parkinson's Disease: A medical and economic study**

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**Rational:** Parkinson's disease (PD) has considerable impact on motor, psychological and social activities and significantly affects the quality of life of patients and their families. To improve the medical care of PD patients, we have developed an educational program specific to PD.

**Aim:** The principal aim of this study was to evaluate our educational Program, comparing the quality of life of PD patients with or without the educational program after 1 year follow-up.