Neuroendocrinology

Lucie Chevrier, Brooke Tata and Nicolas de Roux

INSERM U676, Robert Debré Hospital and Paris Diderot University, Paris, France

New mechanisms Circadian rhythm and metabolism

Two different mechanisms by which circadian clock and metabolism are linked are presented in the two following papers.

Regulation of circadian behavior and metabolism by synthetic REV-ERB agonists

Solt LA, Wang Y, Banerjee S, Hughes T, Kojetin DJ, Lundasen T, Shin Y, Liu J, Cameron MD, Noel R, Yoo SH, Takahashi JS, Butler AA, Kamenecka TM, Burris TP
Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, FL, USA
Nature 2012;485:62–68

Background: The nuclear receptors REV-ERB- α and REV-ERB- β have an important role in regulation of core clock genes expression driving circadian activity and metabolism. Agonists of these receptors could be of interest to regulate circadian rhythm disorders and metabolic disorders.

Methods: The authors synthesized agonists of REV-ERB- α and REV-ERB- β and tested their effects in circadian activity and metabolic regulation.

Results: SR9011 and SR9009 are two synthetic agonists of REV-ERB- α and REV-ERB- β . In vivo, these compounds altered circadian behaviors and the expression of core clock genes in the hypothalamus of mice. The expression of metabolic genes in liver, skeletal muscle and adipose tissue was also altered, which resulted in increased energy expenditure. In obese mice, these agonists decreased body weight.

Conclusion: Agonists of REV-ERB- α and REV-ERB- β appeared to be useful pharmacological tools for the treatment of sleep disorders and metabolic diseases.

REV-ERB- α and REV-ERB- β are nuclear receptors regulating core clock genes. These receptors also play a key role in lipid synthesis. This study proposed a molecular mechanism linking circadian clock and metabolism. In fact, the authors described two new synthetic agonists of REV-ERB- α and REV-ERB- β that disrupted circadian activity and repressed clock gene expression in the hypothalamus. Interestingly, the expression of clock genes in peripheral tissues was not affected by these treatments. The circadian alteration induced by agonists of REV-ERB- α and REV-ERB- β was accompanied by changes in expression of genes involved in metabolism leading to a loss of body weight and to an increase of energy expenditure. These metabolic variations were also seen in obese mice. This study brings new insight about the development of compounds targeting REV-ERB- α and REV-ERB- β and more widely the circadian clock to treat metabolic disorders such as obesity.

Circadian rhythm of redox state regulates excitability in suprachiasmatic nucleus neurons

Wang TA, Yu YV, Govindaiah G, Ye X, Artinian L, Coleman TP, Sweedler JV, Cox CL, Gillette MU
Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL, USA
Science 2012;337:839–842

Background: Circadian rhythms are orchestrated in the suprachiasmatic nucleus (SCN) by a central circadian clock and are controlled by the circadian expression of clock genes. Redox state can regulate the expression of these genes. However, nothing was known about a potential non-transcriptional regulation of redox state on circadian rhythms.

Methods and Results: By ratiometric redox fluorometry by two-photon laser microscopy, the authors demonstrated that in the SCN, the redox state followed an endogenous circadian oscillation for which clock genes are required. Whole cell patch-clamp recording of SCN neurons showed that redox state oscillation could modify the excitability of SCN neurons through modulation of K⁺ channels.

Conclusion: Circadian regulation of redox state by a central circadian clock modulated the excitability of SNC neurons via a non-transcriptional mechanism.

This study is interesting for two main reasons. First, it highlights that, in the suprachiasmatic nucleus (SCN), redox state shows circadian oscillations that are under the control of circadian clock genes, and brings new insight concerning circadian clock and metabolism regulation. The second reason of the interest of this study is the discovery of a novel non-transcriptional mechanism by which the circadian clock can regulate SCN neuron excitability. In fact, the redox state directly influenced the excitability of SCN neurons through modulation of K⁺ channels, indicating that metabolic state variation could be the cause, rather than the result, of neuronal activity. Finally, it seems that at midday, circadian clock favored an oxidized state, leading to the opening of K⁺ channels and to an increase of neuronal activity of SCN neurons. This study focused on the SCN, which is the master circadian clock. However, circadian rhythms affect the entire organism. So it is now of interest to determine if the link between circadian clock and redox state also exists in peripheral tissue.

New mechanisms

GnRH and biosynthesis of neurosteroids

Gonadotropin-releasing hormone stimulates the biosynthesis of pregnenolone sulfate and dehydroepiandrosterone sulfate in the hypothalamus

Burel D, Li JH, Do-Rego JL, Wang AF, Luu-The V, Pelletier G, Tillet Y, Taragnat C, Kwon HB, Seong JY, Vaudry H
Laboratory of G-Protein-Coupled Receptors, Graduate School of Medicine, Korea University, Seoul, Republic of Korea, and Research Institute for Biomedicine (IRIB), Laboratory of Neuronal and Neuroendocrine Differentiation and Communication, Institut National de la Sante et de la Recherche Medicale U982, University of Rouen, Mont-Saint-Aignan, France

jyseong@korea.ac.kr or hubert.vaudry@univ-rouen.fr

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Background: The enzyme hydroxysteroid sulfotransferase (HST) is involved in the biosynthesis of the sulfated neurosteroids, pregnenolone sulfate (Δ^5 PS) and dehydroepiandrosterone sulfate (DHEAS). In the frog, HST is expressed in the magnocellular nucleus and the anterior preoptic area, two hypothalamic regions that are richly innervated by GnRH1-containing fibers. However, nothing is known about the potential regulatory effect of GnRH1 on sulfated neurosteroids synthesis.

Methods and Results: Using double labeling of frog brain slices, the authors showed that GnRH1 fibers are located in close vicinity of HST-positive neurons. Three GnRH receptors were identified. RT-PCR and in situ hybridization were used to localize the three GnRH receptors and in vitro studies allowed the functional characterization of these. Pulse-chase experiments using tritiated Δ^5 P and DHEA as steroid precursors, and 3'-phosphoadenosine 5'-phosphosulfate as a sulfonate moiety donor, showed that GnRH1 stimulated the biosynthesis of Δ^5 PS and DHEAS in frog diencephalic explants.

Conclusion: These data suggest that some of the behavioral effects of GnRH could be mediated by the modulation of sulfated neurosteroids, known to stimulate sexual activity.

This study conducted in frog is interesting for two main points. First, the authors identified and characterized three receptors of GnRH. The wide distribution of the three GnRH receptors in the brain reflected the neurotransmitter, neuromodulator and neuroendocrine roles of GnRH. However, the precise role of each receptor was not yet known. In the pituitary, only GnRHR-1 was expressed, suggesting that this receptor is involved in the regulation of gonadotrope cells by GnRH. GnRHR-1 and

GnRHR-2 were present in the testis and adrenal gland, two peripheral sites of DHEA biosynthesis. It would be interesting to determine if GnRH can modulate biosynthesis of DHEA in these steroidogenic glands. Second, this study brings new insight in neurosteroid synthesis. In fact, it underscores that in the frog, GnRH1 directly stimulates the biosynthesis of two sulfated neurosteroids, pregnenolone sulfate (Δ^5 PS) and dehydroepiandrosterone sulfate (DHEAS). It appears that the GnRH receptors GnRHR-1 and GnRHR-3 are highly expressed in the magnocellular nucleus and the anterior preoptic area, hypothalamic regions wherein GnRH1 fibers are located in close vicinity of HST-positive neurons. So, it can be thought that in the frog, stimulatory effects on GnRH1 on sexual behaviors could be mediated by sulfated neurosteroids.

New mechanism

GnRH-(1-5) controls the migration of GnRH neurons

The metabolite GnRH-(1-5) inhibits the migration of immortalized GnRH neurons

Larco DO, Cho-Clark M, Mani SK, Wu TJ

Program in Molecular and Cellular Biology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA
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Background: GnRH is metabolized to GnRH-(1-5) by the endopeptidase EP24.15. EP24.15 is expressed along the migratory path of GnRH-expressing neurons during development. However, the potential role of GnRH-(1-5) peptide on GnRH neuron migration was unknown.

Methods: The authors used the GN11 cell line as a model of premigratory GnRH-secreting neuronal cells for in vitro studies. The role of GnRH-(1-5) on GN11 migration was determined by a wound-healing assay and RNA interference of GPR173. STAT3 pathway activation was studied by immunocytochemistry and Western immunoblotting.

Results: GnRH-(1-5) inhibited GN11 cell migration and acted via GPR173 and the inhibition of STAT3 pathway.

Conclusion: This in vitro study highlighted the potential regulatory role of GnRH-(1-5) in GnRH neuron migration during development.

Migration of GnRH neurons from the olfactory placode to the hypothalamus is a crucial step to the establishment of the hypothalamic-pituitary-gonadal axis. In fact, defect of GnRH neuron migration due to olfactory agenesis can lead to hypogonadism in Kallmann syndrome. Identification of factors involved in this migration is particularly informative to better understand the pathogenesis of Kallmann syndrome. The authors investigated the potential role of GnRH-(1-5) on the migration of GnRH neurons. The fact that EP24.15 is expressed during development along the migratory path of GnRH neurons and that migrating GnRH neurons secrete GnRH strengthens this hypothesis. In GN11 cells, an established cell line expressing GnRH, the authors showed that GnRH-(1-5) slowed migration of GN11 via the GPCR GPR173 and via the inhibition of the STAT3 pathway. GPR173 is expressed in mouse embryonic nasal tissue from E12.5, when GnRH neurons are migrating along the vomeronasal tract. This study describes an interesting autocrine effect of GnRH-(1-5) on GnRH neuron migration through GPR173 activation that needs to be tested by in vivo investigation.

Changes in hypothalamic expression of the *lin28/let-7* system and related microRNAs during postnatal maturation and after experimental manipulations of puberty

Sangiao-Alvarellos S, Manfredi-Lozano M, Ruiz-Pino F, Navarro VM, Sanchez-Garrido MA, Leon S, Dieguez C, Cordido F, Matagne V, Dissen GA, Ojeda SR, Pinilla L, Tena-Sempere M

Department of Cell Biology, Physiology and Immunology, Faculty of Medicine, University of Cordoba, Cordoba, Spain
susana.sangiao@udc.es

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Background: *lin28* and *lin28b* are RNA-binding proteins known to inhibit the maturation of miRNAs of the *let-7* family. Genome-wide association studies suggest the involvement of *lin28/let-7* in the control of puberty.

Methods: The expression of *lin28* and *let-7* and other components of the regulatory network are studied in the rat hypothalamus during postnatal development, and in models of altered puberty.

Results: Expression of *lin28*, *lin28b* and *c-Myc* was high in neonatal hypothalamus, and decreased during the infantile-to-juvenile transition to reach a minimal level at puberty. *let-7a*, *let-7b*, *mir-132* and *mir-145* have opposite expression profiles. Perturbation of puberty onset by neonatal sex steroid treatment, by photoperiod manipulation or by subnutrition, affected the expression of the *lin28/let-7* system.

Conclusions: The expression of *lin28/let-7* system dramatically changed in the rat hypothalamus during the postnatal maturation and was affected in models of altered puberty, suggesting the involvement of this system in the mechanisms leading to puberty onset.

Recently, miRNAs and their regulators have been involved in the fine regulation of complex biological processes. In this study, the authors provided evidence that miRNAs are involved in the postnatal maturation of the gonadotropic axis. *lin28* was highly expressed in the hypothalamus in the neonatal period and then declined at the puberty, while *let-7* expression varied in the opposite direction. Delayed puberty induced by neonatal administration of sex steroids or modification of the photoperiod was associated to an alteration of the *lin28/let-7* ratio in the hypothalamus. Interestingly, male and female rats did not respond equally to the modification of the photoperiod. Alterations of *lin28/let-7* expression appeared in the juvenile period in males. In females, variations of the *lin28/let-7* ratio appeared in the prepubertal period. In a third model of delayed puberty induced by feeding restriction, *lin28/let-7* ratio expression was not really affected. Although the precise role of *lin28/let-7* system in the hypothalamus needs to be characterized, the data presented here open a new way to understand the postnatal maturation of the gonadotropic axis and how environmental factors, sex steroids or the nutrition status may interfere with this process.

Epigenetic control of female puberty

Lomniczi A, Loche A, Castellano JM, Ronnekleiv OK, Bosch M, Kaidar G, Knoll JG, Wright H, Pfeifer GP, Ojeda SR
Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR, USA

lomniczi@ohsu.edu

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Background: It is well known that not just one gene, but rather a multitude of genes control the onset of puberty. However, the genetic elements and transcriptional regulation coordinating this process for pubertal onset to occur is unknown and underexplored. Further, it does not explain how there can be dynamic plastic changes in gene networks involved at the epigenetic level for pubertal onset.

Methods: The authors injected 5-azacytidine (Aza), a DNA methyltransferase, in juvenile female Sprague-Dawley rats to inhibit DNA methylation in the hypothalamus. With this, authors measured sexual maturation and reproductive function. Next, authors used DNA methylation arrays to discover potential repressor genes at different pubertal stages. Lastly, using the *Kiss-1* gene as a puberty-activating gene prototype, authors carried out various immunohistochemical and quantitative polymerase chain

reaction methods to understand if these repressor genes were expressed in kisspeptin neurons and how they affected kisspeptin immunoreactivity, function, as well as GnRH pulsatility.

Results: The authors discovered that the inhibition of DNA methylation delays puberty through keeping inhibiting genes activated. These repressor genes are a part of a Polycomb Group (PcG) of proteins and particularly two genes, *Eed* and *Cbx7*, whose mRNA levels decreased concomitantly with an increase in DNA methylation at puberty. Lastly, the authors discovered that *Eed* was localized at the promoter of the *Kiss-1* gene and is evicted at the timing of puberty along with changes in chromatin status of *Kiss1*. Overexpression of *Eed* led to compromised reproductive capacity and reduced the number of *Kiss1*-positive neurons in the ARC.

Conclusions: Hypothalamic expression of two repressive PcG genes, *Cbx7* and *Eed*, are important for the repression of pubertal onset. Before puberty begins, expression of these genes decreases coincidentally with increased DNA methylation. *Eed* is located at the PcG promoter and prevents the upregulation of *Kiss1* mRNA before puberty, demonstrating a novel mechanism for the upregulation of *Kiss1* mRNA in the ARC before puberty, involving DNA methylation and the eviction of *Eed* from its promoter.

The timing of puberty is associated with an increase in GnRH release. However, our focus has been mainly on the transynaptic and physiological regulation of GnRH secretion during pubertal onset. How pubertal onset in females is regulated at the epigenetic level and how this transcriptional plasticity can affect the timing of puberty is unknown. These authors investigated, for the first time, how DNA methylation contributes to the regulation of the timing of puberty in females using rats as a model. Inhibition of DNA methylation in the hypothalamus prevented puberty in female rats through the continued activation of a repressive mechanism. Using DNA methylation arrays at different pubertal stages, two genes, *Cbx7* and *Eed*, were discovered as a part of the Polycomb Group (PcG) of transcriptional silencers whose expression decreases before the onset of puberty in association with increased DNA methylation. Using *Kiss1* as a model of a puberty-activating gene to prove that the PcG complex represses the onset of puberty by targeting downstream genes responsible for the excitatory control of GnRH, the authors discovered that the PcG gene *Eed* is associated with the *Kiss1* promoter, preventing accessibility of activating histone marks before pubertal onset. Indeed, overexpression of *Eed* consequently led to the reduction of immunoreactive kisspeptin neurons, decreased GnRH pulsatility, and delayed puberty. Overall, this study unveiled a novel mechanism where epigenetic silencing underlies the timing of pubertal onset. This is important in terms of human fertility and how developmental changes at the epigenetic level can have an impact on reproductive function.

New mechanism

How the gonadotropic axis is inhibited at the end of the infantile period

The decline in pulsatile GnRH release, as reflected by circulating LH concentrations, during the infant-juvenile transition in the agonadal male rhesus monkey (*Macaca mulatta*) is associated with a reduction in kisspeptin content of KNDy neurons of the arcuate nucleus in the hypothalamus

Ramaswamy S, Dwarki K, Ali B, Gibbs RB, Plant TM

Department Obstetrics and Gynecology and Reproductive Sciences, University of Pittsburgh, Magee-Women's Research Institute, Pittsburgh, PA, USA

planttm@mwri.magee.edu

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Background: Puberty in primates is timed by two hypothalamic events — a decline in pulsatile GnRH release occurs in late infancy, which leads to a quiescent phase. Then, in late juvenile development, pulsatile GnRH release is reactivated which allows puberty to be initiated. This phase in late juvenile development is kisspeptin-dependent.

Methods: The authors conducted kisspeptin immunohistochemistry in agonadal male rhesus monkeys during early infancy when GnRH release is high with robust LH concentrations. They compared kisspeptin, neurokinin B, and GnRH immunoreactivity with male rhesus monkeys during the juvenile period when GnRH release is arrested.

Results: Although there was no difference between infant and juvenile male rhesus monkeys in terms of distribution of immunopositive kisspeptin neurons, there were more kisspeptin immunoreactive cell bodies in infant monkeys compared to juvenile monkeys. Interestingly, there was no difference in neurokinin B distribution or GnRH fiber staining intensity.

Conclusion: A reduction in the stimulatory kisspeptin tone to the GnRH neuronal network is required for the switch between the late infancy to juvenile periods in male rhesus monkeys. The reduction in kisspeptin tone guarantees the quiescent hypogonadotropic phase of the prepubertal gonad.

This article raises an interesting question, as we have always been fascinated as to how puberty is activated, but rarely have we investigated the nature of the switch that suppresses GnRH pulsatility after the minipuberty of infancy. This article addresses the question whether the loss of GnRH pulsatility in the infant primate is related to a reduction of kisspeptin in the arcuate nucleus. The findings reveal a remarkable insight into the kisspeptin-neurokinin B-dynorphin (KNDy) neuronal mechanism of GnRH pulsatility. In that first, the GnRH pulse generation that is turned off during infancy is gonad-independent and this inhibits kisspeptin neuronal activity. However, the authors discovered that the number of kisspeptin cell bodies in the ARC was unrelated to the GnRH pulse arrest, suggesting there are two separate mechanisms that regulate GnRH pulsatility and kisspeptin pre- and postpubertal onset. Furthermore, the authors discovered that, during the infant-juvenile transition, kisspeptin content in KNDy neurons significantly decreased along with its pulsatile release, leaving GnRH pulsatility arrested. However, during the juvenile-pubertal transition, kisspeptin content and pulsatility are increased, leading to an increase in GnRH. This is intriguing as these authors suggest that kisspeptin does not in fact play a regulatory role in the timing of puberty, but serves merely as an output to the GnRH pulse generator, suggesting that other mechanisms are required for these two important hypothalamic events pre- and postpubertal onset.

New gene

A new gene in Kallmann syndrome

SEMA3A, a gene involved in axonal pathfinding, is mutated in patients with Kallmann syndrome

Hanchate NK, Giacobini P, Lhuillier P, Parkash J, Espy C, Fouveaut C, Leroy C, Baron S, Campagne C, Vanacker C, Collier F, Cruaud C, Meyer V, Garcia-Pinero A, Dewailly D, Cortet-Rudelli C, Gersak K, Metz C, Chabrier G, Pugeat M, Young J, Hardelin JP, Prevot V, Dode C
INSERM U837, Développement et Plasticité du Cerveau Postnatal, Centre de Recherche Jean-Pierre Aubert, Lille, France

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Background: The genetics of the Kallmann syndrome involves various modes of transmission, including oligogenic inheritance, but mutations have been found in only 35%. Semaphorin-3A is a secreted protein acting as a guidance cue of growing axons. This protein has been shown to be critical for GnRH neuron migration in rodents. In this paper, the authors considered *SEMA-3A* as a candidate gene in Kallmann syndrome.

Methods: Analysis of GnRH neuron distribution in *Nrp1^{sema/sema}* mutant mice that lack a functional semaphorin-binding domain in neuropilin-1, an obligatory co-receptor of semaphorin-3A. 386 unrelated patients were screened for *SEMA-3A* mutations.

Results: *Nrp1^{sema/sema}* showed abnormal development of the peripheral olfactory system and defective embryonic migration of the neuroendocrine GnRH cells to the basal forebrain, which results in increased mortality of newborn mice and reduced fertility in adults. Nonsynonymous mutations were

observed in 24 patients, specifically, a frameshifting small deletion and seven missense mutations leading to low secretion or reduced signaling activity.

Conclusion: Semaphorin-3A signaling insufficiency contributes to the pathogenesis of KS and further substantiates the oligogenic pattern of inheritance in this developmental disorder.

SEMA-3A is a new mutated gene in Kallmann syndrome (KS). With 4 other genes encoding for proteins modulating the *FGFR1* signaling pathway and recently described [1], more than 20 genes have now been linked to congenital hypogonadotropic hypogonadism with or without olfactory bulb agenesis. This study is interesting as it provides a new pathological mechanism to explain defects in olfactory bulb agenesis and therefore GnRH neuron migration. All *SEMA-3A* mutations were heterozygous in KS patients. All but one mutation were very rare. Eight mutations were shown to be loss-of-function, which confirms the model observed in *Sema-3A* knockout mice [2]. However, these heterozygous mutations do not fully explain the KS phenotype. The authors propose that monoallelic mutations in *SEMA3A* are insufficient to induce olfactory bulb agenesis, but probably act in concert with other mutations in genes regulating the semaphorin-3A signaling pathway. A similar conclusion was drawn by Young et al. [3] who also reported a *SEMA-3A* mutation in a family with KS.

New concern

Endocrine disruptor exposure during fetal life and adult obesity

Organizational effects of perinatal exposure to bisphenol-A and diethylstilbestrol on arcuate nucleus circuitry controlling food intake and energy expenditure in male and female CD-1 mice

Mackay H, Patterson ZR, Khazall R, Patel S, Tsirlin D, Abizaid A
Department of Neuroscience, Carleton University, Ottawa, Ont., Canada
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Background: Bisphenol-A (BPA) is a plasticizer and a known endocrine disrupter that can act as an agonist to estrogen receptors and function as an obesogen, possibly causing disrupted hypothalamic energy balance circuitry to the melanocortin system in the arcuate nucleus (ARC) of the hypothalamus.

Methods: Pregnant CD-1 female mice were subjected to environmentally relevant doses of BPA. Offspring were weaned initially onto a normal (AIN93G) diet, then as adults exposed to either a normal or high-fat diet (HFD). During adulthood, metabolic parameters, diet changes, quantification of neurons involved in the regulation of metabolism, and other parameters were measured to study the effects of early life exposure to BPA.

Results: High BPA exposure during early life (in utero and before weaning) in rodents had differential effects between adult male and female mice, as these systems are sexually differentiated. In female offspring, high BPA exposure and high-fat diet during early life led to increased weight gain, adiposity, hyperleptinemia, and masculinization of the melanocortin system, as shown by similar ER- α expression as males. However, males exposed to high BPA and high-fat diet in the same critical period exhibited impaired glucose tolerance and hyperleptinemia, but no change in body mass.

Conclusion: Early life exposure to BPA resulted in sexual dimorphic alterations in the organization and structure of the hypothalamic melanocortin pathway, leading to increased susceptibility to diet-induced diabetes, glucose intolerance and other metabolic impairments.

Bisphenol-A (BPA), a plastic monomer, has structural similarity to 17 β -estradiol, acting as an agonist to the estrogen receptor (ER), and has been implicated as an endocrine disrupter and acts as an obesogen. Of particular interest and concern, BPA exposure can lead to obesity and metabolic dysfunction. The authors posed the question of how early life BPA exposure affects adult metabolic phenotype and hypothalamic energy balance. This is interesting due to the fact that the human population has ubiquitous oral exposure to BPA on a daily basis, leading to higher vulnerability for obesity, metabolic impairments, and sexual dimorphic alterations in the brain if exposed

early in life. This further highlights an important point given that BPA can cross the placental barrier. Furthermore, high doses of exposure to BPA, in combination with dietary challenges, elicits a differential response in the melanocortin pathway in male versus female adults born from mothers subjected to either a high or low dose of BPA. Sexually dimorphic changes in the metabolic phenotype along with differential organization of the melanocortin pathway in the ARC were observed with early life BPA exposure. Indeed, high levels of BPA in association with a high-fat diet masculinized the female brain, predisposing to diet-induced obesity. However, with this the authors raise an important point that there is a degree of ubiquity of BPA in the environment and how to understand the mechanisms in rodent models raises controversy due to the difficulty in experimental design. However, this article overall highlights a major problem that pregnant mothers may predispose their children to diet-induced obesity through environmental estrogens such as BPA.

New hope A new pharmacological target in obesity

Obesity-driven synaptic remodeling affects endocannabinoid control of orexinergic neurons

Cristino L, Busetto G, Imperatore R, Ferrandino I, Palomba L, Silvestri C, Petrosino S, Orlando P, Bentivoglio M, Mackie K, Di Marzo V

Endocannabinoid Research Group, Institute of Cybernetics 'Eduardo Caianiello', and Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Pozzuoli, Italy

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Background: Alterations in energy status, chronic or acute, can change the balance between excitatory and inhibitory synaptic transmission and plasticity in the hypothalamus, which is important for the adaptation of energy metabolism to new homeostatic requirements. The endocannabinoid and cannabinoid receptor type-1 (CB-1) are important modulators of excitatory and inhibitory neurotransmission, are sensitive to leptin, and their tone is altered during obesity.

Methods: To understand if the endocannabinoid system plays an important role in the readjustment of both excitatory and inhibitory inputs into the hypothalamic circuits regulating energy status, the authors used confocal and ultrastructural microscopic analyses and patch-clamp recordings in the leptin-deficient knockout mouse (*ob/ob*) and in mice with diet-induced obesity (DIO).

Results: As endocannabinoids act retrogradely at presynaptic sites to activate CB-1, they inhibit neurotransmitter release and fine tune synaptic transmission. In *ob/ob* mice and mice with DIO, the authors found that orexinergic (appetite-stimulating) neurons undergo a shift from excitatory to inhibitory control via CB-1 inputs. Further, endocannabinoid biosynthesis is increased in these mice, causing an inhibition of orexinergic neurons, leading to hyperphagia, increased body mass, as well as other metabolic problems.

Conclusion: The authors provide initial evidence that increased inhibitory control of orexinergic neurons and their CB1-mediated disinhibition were a consequence of impaired leptin signaling in the arcuate nucleus of the hypothalamus, as the metabolic dysregulation observed was reversed through leptin administration.

Endocannabinoids are important for regulation of energy status amongst other physiological functions where they act to activate retrogradely at presynaptic sites, binding to the cannabinoid receptor 1 (CB1) to inhibit neurotransmitter release and fine tune synaptic transmission. The purpose of this study was to analyze whether CB1 expressing excitatory versus inhibitory inputs to orexin-A neurons in the hypothalamus is affected during obesity and how obesity could thus affect endocannabinoid control of orexin-A neurons. Due to the sensitivity of the endocannabinoid system to leptin, the authors studied leptin-deficient (*ob/ob*) mice and discovered an obesity-related switch of endocannabinoid-mediated retrograde modulation of neuronal activity. With this, leptin deprivation was found to induce changes in synaptic innervation of the hypothalamus,

leading to the remodeling of CB1 expressing inhibitory/excitatory fibers. This study reports a novel example in which endocannabinoid control of excitatory and inhibitory neurotransmission can be greatly changed during obesity due to leptin-dependent reorganization of glutamatergic and GABAergic synapses. The full functional implications of this mechanism need to be further investigated. In conclusion, this article provides novel insight for the development of new drugs against obesity.

New mechanism

How thyroid hormones are involved in the central control of cardiovascular functions

Thyroid hormone is required for hypothalamic neurons regulating cardiovascular functions

Mittag J, Lyons DJ, Sallstrom J, Vujovic M, Dudazy-Gralla S, Warner A, Wallis K, Alkemade A, Nordstrom K, Monyer H, Broberger C, Arner A, Vennstrom B

Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden

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Background: Thyroid hormone is a well-known regulator of metabolic and cardiovascular functions. Molecular mechanisms involved in the central cardiovascular control by thyroid hormone are poorly understood.

Methods and Results: Using a mouse model with a heterozygous mutation of TR α 1 (*Thra1*^{+/-m} mice), the authors identified an unknown population of parvalbumin (pv)-expressing neurons in the anterior hypothalamus which is dramatically decreased (70%) when thyroid hormone signaling is altered. Electrophysiological studies of these cells underscored a temperature and a thyrotropin-releasing-hormone sensitivity. Ablation of parvalbuminergic neurons resulted in hypertension and temperature-dependent tachycardia.

Conclusions: The authors identified a novel population of hypothalamic neurons expressing pv that required thyroid hormone signaling for proper development and that are involved in blood pressure. These findings provided a connection between hypothyroidism and cardiovascular disorders.

The central regulation of cardiovascular functions by thyroid hormones was suggested but the anatomical site was unknown. This study is particularly interesting because it leads to the identification of a population of parvalbumin (pv) neurons in the anterior hypothalamus involved in the central regulation of cardiovascular functions by thyroid hormone. In hypothyroid mice due to impaired thyroid hormone signaling, this pv cell population was reduced because of a developmental defect. Electrophysiological studies identified at least four subpopulations of parvalbuminergic cells in the anterior hypothalamus, but it was not possible to better differentiate these subpopulations. Specific ablation of these pv neurons in the anterior hypothalamus by a sophisticated method using stereotaxic injection of a novel conditionally neurotoxic adeno-associated virus into the anterior hypothalamus of pv-Cre transgenic mice indicated that pv neurons regulate blood pressure directly by the autonomic nervous system rather than an endocrine system. Even if the molecular mechanisms of central regulation of cardiovascular function by thyroid hormone need to be specified, these data may have an important clinical implication for the monitoring of the cardiovascular functions in hypothyroidism patients.

New mechanism

The role of glial cells in the control of TSH expression

Coordination of hypothalamic and pituitary T₃ production regulates TSH expression

Fonseca TL, Correa-Medina M, Campos MP, Wittmann G, Werneck-de-Castro JP, Arrojo EDR, Mora-Garzon M, Ueta CB, Caicedo A, Fekete C, Gereben B, Lechan RM, Bianco AC

Division of Endocrinology, Diabetes and Metabolism, Miller School of Medicine, University of Miami, Miami, FL, USA
J Clin Invest 2013;123:1492–1500

Background: Type II deiodinase (D₂) activates thyroid hormone by converting thyroxine (T₄) to 3,5,3'-triiodothyronine (T₃). This allows plasma T₄ to signal a negative feedback loop that inhibits production of thyrotropin-releasing hormone (TRH) in the mediobasal hypothalamus (MBH) and thyroid-stimulating hormone (TSH) in the pituitary.

Methods: Two mouse strains with pituitary- and astrocyte-specific D₂ knockdown were developed by crossing D₂-lox/lox mice with mice specifically expressing the *Cre* recombinase in thyrotrope or astrocytes. The homeostasis of the hypothalamo-pituitary thyroid was then investigated.

Results: Deletion of D₂ in thyrotropes (pit-D₂ KO) resulted in high serum T₄, normal serum T₃ leading to euthyroidism, and a 3-fold elevation in serum TSH levels with a 40% reduction in biological activity. This decrease of the TSH bioactivity was the result of the elevated serum T₄ that increased D₂-mediated T₃ production in the MBH, thus decreasing *Trh* mRNA by a negative feedback on D₂ expressed in tanycytes. D₂ deletion in astrocytes did not affect the HPT.

Conclusion: Despite near-complete loss of brain D₂, tanycyte D₂ was preserved in astro-D₂ KO mice at levels that were sufficient to maintain both the T₄-dependent negative feedback loop and thyroid economy. Taken together, these data demonstrated that the hypothalamic-thyroid axis is wired to maintain normal plasma T₃ levels, which is achieved through coordination of T₄-to-T₃ conversion between thyrotrophs and tanycytes.

This paper describes an interesting new loop of regulation of the thyroid axis. This loop involves interaction between neurons and glial cells in the hypothalamus. A functional interaction between tanycytes and TRH bioactivity was already known. Indeed tanycytes have the capacity to cleave proTRH in active TRH peptides. Here, the authors showed the link between D₂ expression in tanycytes and *Trh* expression in neurons. This results from the regulation of T₃ production by D₂ expressed in tanycytes. The second main result showed that TSH bioactivity was decreased in mice with a conditional deletion of D₂ in thyrotropes. This decrease of TSH bioactivity is due to an alteration in TSH glycosylation resulting from the low expression of *Trh* in the hypothalamus. This increase in TSH levels with reduced TSH bioactivity has a neutral effect on thyroidal activity. Further work will define whether this role of tanycytic D₂ in the feedback regulation mechanism on the hypothalamo-pituitary regulation of the thyrotropic axis in mouse is also relevant in humans.

New mechanism

Role of AGRP hypothalamic networks in hunger

Deconstruction of a neural circuit for hunger

Atasoy D, Betley JN, Su HH, Sternson SM

Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA, USA

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Background: Hunger is a complex behavioral state that elicits intense food seeking and consumption. Activation of starvation-sensitive AGRP neurons evokes voracious eating even in well-fed mice. Inversely, ablation of AGRP neurons results in aphagia. However, synaptic interactions of AGRP neurons with other cell populations need to be elucidated.

Methods: The authors used optogenetic and pharmacogenetic techniques to map neuronal interactions with AGRP neurons.

Results: An inhibitory circuit with paraventricular hypothalamus (PVH) neurons substantially accounted for acute AGRP neuron-evoked eating. Within the PVH, the authors found that AGRP neurons target and inhibit oxytocin neurons in the forebrain, a small population that is selectively lost in Prader-Willi syndrome, a condition involving insatiable hunger.

Conclusion: By developing strategies for evaluating molecularly defined circuits, the authors show that AGRP neuron suppression of oxytocin neurons is critical for evoked feeding. These experiments reveal a new neural circuit that regulates hunger state and pathways associated with overeating disorders.

This paper reports a highly detailed analysis of three neuronal networks involving AGRP neurons. These results were mainly based on the optogenetic approach, which is a very powerful technique to characterize the neuronal network. One of these networks, involving AGRP neurons in feeding behavior, relies on the inhibition of POMC neurons which are more involved in longer-term rather than acute feeding responses. A second network inhibits paraventricular hypothalamic neurons through synaptic contacts with AGRP neurons. This second network controls acute AGRP neuron-evoked feeding. The fact that AGRP neurons exert an inhibitory effect on oxytocin neurons located in the paraventricular hypothalamus represents a new pathway in the control of hunger. This result confirms the known function of the central oxytocin in controlling feeding. In addition to the description of this new pathway, the authors suggest that the insatiable hunger in patients with Prader-Willi syndrome is due to the loss of oxytocin neurons reported in this syndrome. Altogether, the characterization of this new circuit that regulates hunger suggests new therapeutic targets in feeding disorders.

New mechanism

How ghrelin bypasses the blood-brain barrier to modulate hypothalamic neuron plasticity

Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons

Schaeffer M, Langlet F, Lafont C, Molino F, Hodson DJ, Roux T, Lamarque L, Verdier P, Bourrier E, Dehouck B, Banerjee JL, Martinez J, Mery PF, Marie J, Trinquet E, Fehrentz JA, Prevot V, Mollard P

Centre National de la Recherche Scientifique, Unité Mixte de Recherche 5203, Institut de Génétique Fonctionnelle, Montpellier, France

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Background: The neurons of the arcuate nucleus (ARH) are critical in the sense that they must be able to rapidly sense and integrate peripheral signals to maintain metabolic homeostasis.

Methods: The authors developed a novel technique of in vivo multiphoton microscopy along with fluorescently labeled ghrelin to demonstrate in real-time the extravasation of ghrelin from the periphery into the ARH in order to understand the mechanism how blood-borne molecules can rapidly access their target neurons by bypassing the blood-brain barrier.

Results: The authors discovered that ghrelin rapidly binds neuropeptide-Y and pro-opiomelanocortin (POMC) neurons in the near vicinity of the fenestrated capillaries of the median eminence (ME), whose vessels are connected to the ventromedial ARC (vmARH). With this, the authors observed plasticity in the system, in that ghrelin-positive neurons varied with feeding status.

Conclusions: The hypothalamus, in particular the ARH, is able to not only directly sense peripheral signals, but also modify energy status in a nutrient-dependent plastic manner.

Satiety signals fluctuate on an ultradian rhythm where peripheral food intake signals must be rapidly sensed centrally for homeostatic regulation of feeding responses. However, the mechanisms by which peripheral blood-borne molecules circumvent the blood-brain barrier and reach the arcuate nucleus (ARH) to enable rapid homeostatic regulation of energy status is currently underexplored. The

authors developed a novel tool to study this mechanism using in vivo multiphoton microscopy in real time with tractable bioactive ghrelin. They discovered that circulating fluorescently labeled ghrelin can extravasate through the fenestrated capillaries of the median eminence (ME), whose vessels project to the ARH, to rapidly bind to their target sites and centrally regulate metabolic signals in a plastic manner. This article is novel and important in the aspect of not only the development of a new technology to study how peripheral metabolic molecules can be sensed centrally in a rapid manner, but also how this process for metabolism is plastic, which is required for the dynamic sensing of the hypothalamus to external feeding patterns to be able to adapt in a plastic fashion. This is of utmost importance when considering clinical pathologies such as obesity and diabetes. In all, this study opens new doors for both the study of how peripheral metabolic signals can be sensed rapidly and in a plastic manner via the ME, and also unveils potential access routes for other molecules that integrate between the periphery and the brain.

References

1. Miraoui H, Dwyer AA, Sykiotis GP, Plummer L, Chung W, Feng B, et al: Mutations in FGF17, IL17RD, DUSP6, SPRY4, and FLRT3 are identified in individuals with congenital hypogonadotropic hypogonadism. *Am J Hum Genet* 2013;92:725–743.
2. Cariboni A, Davidson K, Rakic S, Maggi R, Parnavelas JG, Ruhrberg C: Defective gonadotropin-releasing hormone neuron migration in mice lacking SEMA3A signalling through NRP1 and NRP2: implications for the aetiology of hypogonadotropic hypogonadism. *Hum Mol Genet* 2011;20:336–344.
3. Young J, Metay C, Bouligand J, Tou B, Francou B, Maione L, et al: SEMA3A deletion in a family with Kallmann syndrome validates the role of semaphorin 3A in human puberty and olfactory system development. *Hum Reprod* 2012;27:1460–1465.