Neuroendocrinology

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The choice of papers in neuroendocrinology for the Yearbook is a cruel dilemma. The borders of this field are confusing, in particular in clinics. The hypothalamus is the place of central regulation of all endocrine axes as well as metabolic homeostasis. Neuroendocrine dysregulation leading to endocrine disorders is thus rarely detailed without complete analysis of peripheral hormones. Most of these clinical papers are thus reported in other chapters of the Yearbook. This is why we focused our attention on articles which mainly report fundamental research and are therefore considered as being pretty far away from human clinics. Some of these new concepts will probably never be confirmed in human physiology but others represent really new original findings with some rapid application in humans.

New mechanism: maternal feeding determines the risk of obesity in offspring

Maternal high-fat diet and fetal programming: increased proliferation of hypothalamic peptide-producing neurons that increase risk for overeating and obesity

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Background: Recent studies have demonstrated the close association between dietary fat or circulating lipids with stimulation of hypothalamic peptides involved in the control of food intake and body weight. In this study, Chang et al. have tested the hypothesis that a fat-rich diet during pregnancy alters the regulation and synthesis of these peptide systems in utero, leading to postnatal neuronal changes in the offspring that persist after the diet and have long-term consequences.

Methods: Pregnant Sprague-Dawley rats were fed from embryonic day 6 (E6) to birth with a high-fat diet (HFD) or a balanced diet (BD). At birth, offspring were suckled by their own dams and maintained on their respective diets until they were killed at different postnatal ages. A 3rd group, referred to as HFD-BD, was composed of offspring which were removed from their HFD dams and suckled by BD dams that had no prior exposure to the HFD. A 4th BD-HFD group was composed of BD offspring exposed to HFD after birth. The offspring were killed at different postnatal ages (P) by rapid decapitation.

Results: The offspring of dams on a HFD versus BD from E6 to P15 showed an increase in caloric intake, body weight, leptin and insulin, a stronger preference for fat, elevated TG levels and an increased expression of orexigenic peptides, galanin, enkephalin and dynorphin in the paraventricular nucleus and orexin and melanin-concentrating hormone in the perifornical lateral hypothalamus. The increased density of these peptide-expressing neurons was also observed in HFD offspring fostered at birth to BD dams, suggesting events that might be occurring in utero. During gestation, HFD stimulated the proliferation of neuroepithelial and neuronal precursor cells of the embryonic hypothalamic third ventricle. It also stimulated the proliferation and differentiation of neurons and their migration toward hypothalamic areas.

Conclusion: An increase in neurogenesis was observed in offspring from dams fed with HFD. This increase was closely associated with a marked increase in lipids in the blood. It may play a role in producing the long-term behavioral and physiological changes observed in offspring after weaning, including an increase in food intake, preference for fat and higher body weight.

This study reproduces previous studies showing that perinatal HFD increased several hypothalamic parameters involved in the regulation of food intake and fat mass. This difference is only apparent after weaning in offspring exposed prenatally to HFD suggesting that the prenatal period is a critical time in programming expression of these peptides in the hypothalamus. The increase in peptide expression in the hypothalamus was site-specific. The most interesting finding of this paper is the proposed mechanism leading to this increased expression. Rather than an increase in gene expression in specific neurons, they observed an increase in hypothalamic neurons expressing these peptides. This increased neurogenesis related to prenatal diet has never been reported and seems to be site-specific as it did not occur in the arcuate nucleus. It is related to an increase in proliferation rather than an increase in cell survival. Analysis of several markers of cell differentiation indicates that HFD also induces differentiation in post-mitotic neurons. Persistent neurogenesis in the postnatal brain is now well known and mainly occurs in the subventricular zone and dentate gyrus. Similar postnatal neurogenesis is described in the hypothalamus as well. It would be interesting to study whether postnatal neurogenesis is also enhanced by prenatal feeding with HFD and therefore highly determined by prenatal environment.

Finally, this study led us to revise the concept of the transmission of a complex genetic trait such as obesity. The role of environmental factors in obesity is well known. This study suggests that it is necessary to consider maternal feeding during pregnancy as a risk factor of obesity as well. The most dramatic consequence revealed by this study is the irreversible effect of such prenatal maternal feeding on hypothalamic neurogenesis of the offspring.

Concepts revised: the composition of what you eat determines apoptosis of hypothalamic neurons

High-fat diet induces apoptosis of hypothalamic neurons

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Background: Recent studies have shown that consumption of dietary fats promotes hypothalamic resistance to leptin and insulin. This resistance is the consequence of diet-induced activation of inflammation in the hypothalamus. Since inflammation can lead to the activation of an apoptotic process, the authors evaluated the effect of high-fat feeding on the induction of apoptosis of hypothalamic cells.

Methods: Adult male rats were submitted to a high-fat diet or control diet for 8 weeks, after which they received intracerebroventricular leptin or insulin to evaluate resistance to those peptides. At the end of the experimental period, the animals were killed by decapitation and hypothalami were extracted for determination of protein expression and immunohistochemistry.

Results: After 8 weeks of HFD, all HFD-fed rats showed functional resistance to insulin and leptin. Gene expression analysis revealed an increase of 57% in 84 apoptosis-related genes in the hypothalamus. Dietary fats induce apoptosis of neurons and a reduction of synaptic inputs in the arcuate nucleus and lateral hypothalamus. This effect is dependent upon diet composition, and not on caloric intake, since pair-feeding is not sufficient to reduce the expression of apoptotic markers. The Toll-like receptor 4 protects cells from further apoptotic signals.

Conclusion: In diet-induced inflammation of the hypothalamus, TLR4 exerts a dual function, on one hand by activating proinflammatory pathways that play a central role in the development of resistance to leptin and insulin, and on the other hand by restraining further damage by controlling the apoptotic activity.

In this paper, Moraes et al. show that high-fat feeding of rats induced an inflammation in the hypothalamus followed by neuronal apoptosis in the arcuate nucleus. The inflammatory process is mediated via activation of the toll-like receptor 4 (TLR4) leading to cytokine secretion such as $\text{TNF}\alpha$ in the hypothalamus. This increase in $\text{TNF}\alpha$ probably triggers neuronal apoptosis in the arcuate nucleus.

This mechanism is original and must be compared to the induction by HFD of neurogenesis in the hypothalamus. On one hand, there is an increase in neurons and, on the other, HFD induced neuronal apoptosis. Different mechanisms control these two effects which occur in different parts of the hypothalamus. It is interesting to note that such inflammation was not described in the offspring of HFD dams described in the Chang paper (see above) even though triglyceride levels were high at birth. It will be important to investigate the proposed dual role for TLR4 in the hypothalamus, participating in the balance between inflammation and cell survival. Recent studies have shown that insulin and leptin resistance in extra-hypothalamic brain regions may relate to Alzheimer's disease and depression. It will be exciting to evaluate the participation of TLRs and high-fat feeding in these contexts [1].

New hope: NF- κ B signaling pathway, a new target in obesity treatment

Hypothalamic IKK β /NF- κ B and ER stress link overnutrition to energy imbalance and obesity

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Cell 2008;135:61–73

Background: Chronic metabolic inflammation can be induced by overnutrition, but whether it affects neuronal regulation of energy balance and lead to related diseases is unknown.

Methods and Results: In this study, the authors show that high levels of IKK β /NF- κ B, a major regulator of inflammation, are detected in hypothalamic neurons. Under normal nutritional conditions the IKK β /NF- κ B pathway remains inactive. However, it is strongly activated by overnutrition, due at least partly to an increase in endoplasmic reticulum stress. Chronic activation of hypothalamic NF- κ B by a constitutively active IKK β blunts central insulin/leptin signaling and actions, and site or cell-specific suppression of IKK β in the whole brain or in the mediobasal hypothalamus only or even more specifically in the hypothalamic agouti-related protein (AgRP) neurons can prevent glucose intolerance and obesity onset. Hypothalamic activation IKK β /NF- κ B leads to an increase in SOCS3 gene expression, a core inhibitor of insulin and leptin signaling.

Conclusion: These results show that the IKK β /NF- κ B pathway in hypothalamic AgRP neurons is a key mechanism for energy imbalance underlying obesity and that suppressing IKK β /NF- κ B in hypothalamic neurons represents a strategy for counteracting obesity and associated diseases induced by overnutrition.

While chronic inflammation in peripheral metabolic tissues is generally considered a consequence of overnutrition and obesity, this study provides new insights showing that a metabolic inflammatory pathway can be activated in the brain under high-fat diet and participates in insulin and leptin insensitivity, therefore promoting obesity onset and associated diseases. The authors show that this vicious circle can, however, be blocked in mice in which IKK β /NF- κ B signaling is specifically inhibited in hypothalamic orexigenic AgRP neurons (which also express NPY) and therefore prevent them from obesity. Activation of IKK β by phosphorylation induces phosphorylation of its substrate I κ B α , and subsequent proteosomal degradation of I κ B α releases NF- κ B to translocate into the nucleus where it mediates the transcription of its target genes. These neurons express SOCS3, which is a critical inhibitor for both insulin and leptin in the hypothalamus. This paper shows that IKK β /NF- κ B promotes SOCS3 expression and therefore this upregulation may be the cause of the leptin and insulin resistance in obese mice. This encouraging result could lead to the development of novel anti-inflammatory treatment to prevent overnutrition-induced activation of hypothalamic IKK β /NF- κ B. Because IKK β /NF- κ B normally remains inactive in the CNS, suppressing IKK β /NF- κ B in the hypothalamus is likely a safe approach.

MeCP2, a key contributor to neurological disease, activates and represses transcription

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Science 2008;320:1224–1229

Background: Rett syndrome is a neurodevelopmental disorder caused by the inactivation of the transcriptional repressor methyl-CpG binding protein 2 (MeCP2). Many phenotypes observed in patients with MeCP2 mutations suggest hypothalamic dysfunctions such as sleep abnormalities, abnormal response to stress as measured by increased cortisol levels, episodes of heightened anxiety and growth deceleration. MeCP2 invalidation in mice reproduces many phenotypes seen in patients with Rett syndrome. In this study, the authors sought to examine gene expression patterns in the hypothalamus that either lack or overexpress MeCP2.

Methods: Microarray was performed on the hypothalamic RNA of mice at 6 weeks of age bearing a MeCP2 null allele or overexpressing MeCP2.

Results: In both models, MeCP2 dysfunction induces changes in the expression of thousands of genes but unexpectedly the majority of genes (~85%) appeared to be activated by MeCP2. MeCP2 binding was confirmed on six genes selected by the authors. Furthermore, an association of MeCP2 with the transcriptional activator CREB1 was observed at the promoter of an activated target but not on a repressed gene.

Conclusion: These results suggest that MeCP2 regulates the expression of a wide range of genes in the hypothalamus and it can both function as an activator and a repressor of transcription.

Deletion of Mecp2 in Sim1-expressing neurons reveals a critical role for MeCP2 in feeding behavior, aggression, and the response to stress

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Neuron 2008;59:947–958

Background: The paper reported above demonstrated that MeCP2 can function as an activator as well as a repressor of gene expression in the hypothalamus. This result was unexpected and opened new horizons to map the neuroanatomic origins of the complex neuropsychiatric behaviors observed in patients with Rett syndrome (RTT). In this second paper, the authors have tried to uncover the endogenous functions of MeCP2 in the hypothalamus.

Methods: MeCP2 was specifically invalidated in Sim1-expressing hypothalamic neurons using Cre-loxP strategy.

Results: Loss of MeCP2 in Sim1-expressing neurons led to increased physiological response to stress similar to what is seen upon MeCP2 dysfunction in the entire brain. Unexpectedly, MeCP2 conditional knockout (CKO) mice were aggressive, hyperphagic and obese. MeCP2 CKO mice express high levels of Npy in the dorsomedial nucleus of the hypothalamus and hypothalamic expression of 2 MC4R downstream targets is decreased.

Conclusion: This study has demonstrated that deletion of MeCP2 in Sim1-expressing neurons results in a subset of phenotypes observed in RTT and related MeCP2 disorders. It also revealed a role for MeCP2 in the MC4R signaling pathway.

Rett Syndrome (RTT) is an autism spectrum disorder caused by loss of function of the methyl-CpG-binding protein 2. The many phenotypes that are seen in patients with RTT stem from MeCP2 dysfunction in neurons throughout the brain. Previous transcriptional profiling studies comparing brain tissue from MeCP2-null and wild-type (WT) mice revealed subtle differences in gene expression. Chahrour et al. thus decided to focus their attention on the hypothalamus since a number of RTT phenotypes (anxiety, growth deceleration, sleep-wake rhythm disturbance and autonomic abnor-

malities) could be attributed to hypothalamic dysfunction. MeCP2 was originally described as a transcriptional repressor for its capacity to bind methylated DNA. The transcriptional analysis performed by Chahrour et al. clearly revealed that the majority of genes (2,184) differentially expressed between MeCP2 null and WT mice appeared to be activated by MeCP2 at least in the hypothalamus. The transcriptional changes suggest that the duplication phenotype is due to MeCP2 gain of function (hyper-morph), rather than loss of function. The hypothesis that MeCP2 represses a repressor from those 2,184 genes is unlikely as there was no such repressor among the MeCP2 repressed genes. The study of Chahrour et al. thus revealed for the first time a dual activity of MeCP2, its interaction with CREB1 protein on promoter of activated genes and its involvement in the hypothalamus physiology. Thus, patients with RTT will have to be treated differently than patients with *MECP2* duplications. The finding that MeCP2 regulates a large number of genes, at least in the hypothalamus, suggests a need for therapeutic strategies that focus on restoring neuronal function rather than restoring the activity of individual gene products affected by MeCP2 dysfunction.

The second paper revealed a critical role for MeCP2 in feeding behavior, aggression and response to stress. Although the increased response to stress is a well-known phenotype of RTT, feeding problems have been reported in RTT patients but they are not included in the classical phenotype of RTT. MeCP2 CKO mice showed higher serum corticosterone after 30 min of restraint when compared to WT mice. This altered corticosterone response to stress might reproduce the increased urinary cortisol excretion in patients with RTT. This point is very interesting as corticotrophin-releasing factor (CRF) is produced by paraventricular neurons and its expression is regulated by cAMP-protein kinase A pathway and therefore possibly by CREB1 protein. Altogether, these results indicate that MeCP2 might be involved in the normal regulation of CRF synthesis in the hypothalamus.

The second unexpected result was the obesity associated with hyperphagia and disruption of MCR4 signaling. Hyperphagia and obesity are characteristics of mice that have disrupted MC4R signaling. Fyffe et al. found a decreased expression of *Bdnf* in the PVN of MeCP2 CKO mice. BDNF was recently associated with food intake and its gene expression decreased in MCR4 invalidated mice. It is thus likely that MeCP2 directly regulates *Bdnf* expression.

A free-interval after birth is a characteristic of RTT. These 2 papers revealed a fundamental role for MeCP2 in some hypothalamic functions which mature after birth. Therefore, these papers highlight the possible role of MeCP2 in the postnatal maturation of the hypothalamus.

New gene and new paradigm: far away does not mean unrelated

Regulation of a remote *Shh* forebrain enhancer by the *Six3* homeoprotein

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Background: In humans, SHH haploinsufficiency is responsible for holoprosencephaly (HPE), an anterior midline defect. SHH expression above a critical threshold in the forebrain is very important, but very little is known about the upstream regulators involved.

Methods and Results: In this study, the authors report a rare nucleotide variant located 460 kb upstream of SHH in a HPE patient, and show that this mutation results in the loss of *Shh* brain enhancer-2 (SBE2) in the hypothalamus of transgenic mouse embryos. The SBE2 sequence was then screened for putative DNA-binding protein using DNA affinity-capture assay, and *Six3* and *Six6*, both members of the homeodomain-containing protein family, were identified as candidate regulators of *Shh* transcription. *Six3* has a reduced binding affinity for the mutant SBE2 sequence compared to the wild-type and *Six3* with HPE-causing mutations is unable to bind and activate SBE2.

Conclusion: These data bring evidence of a direct link between *Six3* and *Shh* regulation during normal forebrain development and in the pathogenesis of HPE.

The sonic hedgehog *Shh* encodes a signal that is instrumental in patterning the early embryo. Enhancer sequences play a major role in regional expression of *Shh* in the mouse central nervous

system and previous studies of functional *Shh* regulatory elements have already identified 6 enhancers within a 500-kb interval surrounding the *Shh* gene. Through the identification of single nucleotide variation in the highly conserved SBE2 enhancer leading to an impaired regulation of *SHH* by Six3 in the forebrain causing holoprosencephaly, this article outlines the importance of researching regulatory SNPs modulating gene expression in rare and common diseases and brings a better understanding of the mechanisms underlying *Shh* regulation by Six3 and therefore the molecular mechanism of HPE due to Six3 mutations. The phenotype of this case included a moderate fusion of the hypothalamus and basal ganglia. The altered expression of *SHH* observed in this case should thus be investigated in regard of the recent involvement of *SHH* pathway in hypothalamic patterning [2].

This article is an additional example of the growing new concept that analysis of a candidate region in genetic diseases with Mendelian transmission cannot be restricted to the coding sequence.

New hormone: during the evolution, reproduction of all species is determined by a 'Kiss'

Molecular evolution of multiple forms of kisspeptins and GPR54 receptors in vertebrates

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Endocrinology 2009;150:2837–2846

Background: The *KiSS-1/KiSS-1* receptor (GPR54) system plays a fundamental role in the regulation of the central control of reproduction. While *KiSS-1/GPR54* pair has mainly been characterized in mammals, little is known about this system in non-mammalian vertebrates and about the molecular evolution history of these proteins. In this study, the authors have cloned Kisspeptin and GPR54 cDNAs from a variety of vertebrate species to analyze molecular evolution.

Methods: The authors based their initial analysis on Blast search information from human, zebra fish and *Xenopus laevis* Kisspeptin-10. For GPR54, they did a blast search using human and bullfrog GPR54.

Results: Blast search revealed two forms of kisspeptin genes in fish, *KiSS-1* and *KiSS-2* while *xenopus* possesses three forms of kisspeptin genes, *KiSS-1A*, *KiSS-1B* and *KiSS-2*. The non-mammalian *KiSS-1* gene was found to be the ortholog of the mammalian *KiSS-1* gene while the *KiSS-2* gene is a novel form encoding a C-terminal amidated dodecapeptide. Two isoforms of the GPR54 gene were found in fish, while *xenopus* carry three receptor isoforms. Kisspeptins and GPR54 were abundantly expressed in the *xenopus* brain, notably in the hypothalamus, suggesting that these ligand-receptor pairs play neuroendocrine and neuromodulatory roles. GPR54 isoforms exhibit differential sensitivity towards *KiSS-1*- or *KiSS-2*-derived peptides, indicating differential ligand selectivity.

Conclusion: The authors concluded that two lineages of kisspeptin and GPR54 genes exist in vertebrates.

Lee et al. report in their paper that non-mammalian vertebrate *KiSS* and *GPR54* gene isoforms originate from a common ancestral gene which has been further duplicated. The *KiSS-1*-like gene was designated *KiSS-2* because it encodes a peptide that shares a conserved C-terminal pentapeptide with the peptide encoded by *KiSS-1*. *KiSS-2* peptides are able to activate GPR54. The evolutionary history of *KiSS-1* and *GPR54* is quite long. The authors propose that duplication of an ancestral kisspeptin gene took place early during vertebrate evolution with an additional round of gene/chromosome duplication in the amphibian. The absence of *KiSS-2* in the higher mammalian species such as rodents or primates indicates that *KiSS-2* probably degenerated after divergence of mammalian species.

This paper also reports the characterization of the mature endogenous KiSS-2 peptide in the brain of *X. laevis*. Its structure is composed of 12 residues resulting probably from maturation of the propeptide by a proconvertase at an arginine residue located 2 amino acids upstream of the mature peptide N-terminal end. The exact structure of the KiSS-1 peptide in the brain remains unknown, and it is quite difficult to speculate about this structure as there is no consensus sequence that may lead to cleavage by a proconvertase immediately upstream of the N-terminal end of primate and rodent Kp-10.

All Kisspeptin and GPR54 isoforms are expressed in the hypothalamus suggesting a neuroendocrine function for all of them. The Kisspeptin form involved in the regulation of the gonadotropic axis remains unknown in fish. This very complex regulation of the Kisspeptin system in fish must be compared to the GnRH system. Three GnRH isoforms have been described in several vertebrate species as well as 2 or 3 GnRH receptor isoforms. Although two types of GnRH receptors were characterized in primates, GnRHR2 is a pseudogene in humans. Its physiological role, if any, is not known. The hypothesis that each kisspeptin/GPR54 pair is specific to one type of GnRH neuron needs to be explored.

Discovery of potent kisspeptin antagonists delineate physiological mechanisms of gonadotropin regulation

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Background: Kisspeptin activation of GPR54 was recently described as the most powerful GnRH secretagogue. Natural loss of function mutations of GPR54 as well as genetic invalidation in mice of Kisspeptin and GPR54 have revealed that this Kisspeptin/GPR54 activity is necessary for the reactivation of the gonadotropic axis at puberty as well as for normal reproduction in adult. However, the effects of Kisspeptin functional inactivation has never been reported in adult animals bearing no genetic defects.

Method: The authors developed kisspeptin antagonists by in vitro and in vivo studies to determine the direct role of kisspeptin neurons in the central regulation of the gonadotropic axis in absence of any genetic defects. Various models were used: female rhesus monkey, female ewes and adult male rodents (rat or mice).

Results: One kisspeptin antagonist, Peptide 234, was defined by its capacity to totally block kp-10-induced IP intracellular accumulation. The IC_{50} of the peptide 234 is 7 nM. It blocked Kp-10 stimulation of GnRH neuron firing as well as Kp-10 stimulation of GnRH pulsatile secretion in pubertal female rhesus monkey. It inhibited kp-10-induced LH release in male rats and mice and the LH increase after castration in sheep, rats and mice. However, basal LH was kept within the normal range.

Conclusion: The experiments with Kisspeptin antagonists provide an interesting contribution to the explanation of the neuroendocrine role of Kisspeptin in the regulation of the hypothalamic-pituitary-gonadal axis, namely their fundamental role in pubertal onset and their role as targets for the negative feedback actions of steroids. These antagonists could be a novel approach in therapeutic treatments of reproductive disorders.

This is the first published report of a direct study of kisspeptin function using kisspeptin antagonists. The results confirm the essential role of kisspeptin in the central regulation of the gonadotropic axis and puberty onset, and give some supplemental data. Surprisingly, the kisspeptin antagonist did not inhibit basal LH, suggesting that other factors regulate basal GnRH and LH secretion in all studied species. These results are discordant with the very low LH and FSH basal levels observed in some patients with loss of function mutation of GPR54. These results might highlight differences between species in the regulation of basal levels of LH and FSH, or indicate that GPR54 has an effect on LH and FSH release independent of Kisspeptin. The more severe gonadotropic deficiency in *GPR54^{-/-}* mice when compared to *KiSS-1^{-/-}* mice supports the hypothesis of a Kp-10-independent activation of GPR54. This study suggests that the Kp-10 antagonist could be a novel therapeutic approach in reproductive disorders associated with abnormal LH pulse frequency or amplitude such as infertility, poly-

cystic ovarian syndrome, endometriosis, uterine fibroids, excessive menstrual bleeding, delayed and precocious puberty, and breast, prostatic, and ovarian cancers. Potent and specific kisspeptin antagonists offer potential novel treatments for these conditions.

Kisspeptin-54 at high doses acutely induces testicular degeneration in adult male rats via central mechanisms

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Background: KiSS-1/GPR54 inactivation results in gonadotropic deficiency and this system is a key regulator of pubertal maturation and reproduction. Acute central or peripheral administration of kisspeptin to rodents or primates stimulates the gonadotropic axis. Secondary effects of this injection have not been studied with regard to testicular damage. The authors evaluated the consequences of high doses of Kisspeptin-54 (Kp-54) sustained injection in the rat.

Methods: Histology analyses and hormonal dosage after Kp-54 administration in adult male Wistar rats were made. Different conditions were tested: subcutaneous administration from 6 h up to 3 days; single subcutaneous administration of 0.5 to 50 nmol Kp-54, and before or after pre-administration of a GnRH-receptor antagonist (cetrorelix). The results were compared to the testicular histology in rats after intracerebroventricular injections of 5 nmol Kp-54 to determine whether the cause of testicular degeneration is of central origin or not.

Results: Continuous subcutaneous administration of Kp-54 caused testicular degeneration after only 12 h, when gonadotropins were still markedly high, suggesting that the degeneration is independent of the desensitization of the HPG axis to Kp-54. Furthermore, a single subcutaneous injection of Kp-54 caused dose-dependent testicular degeneration. Thus, continuous Kp-54 administration is not required to cause testicular degeneration. Pretreatment with cetrorelix blocked kisspeptin-induced testicular degeneration, and a single intracerebroventricular injection of Kp-54 caused testicular degeneration, suggesting it is GnRH-mediated.

Conclusion: The mechanisms of Kp-54-induced testicular degeneration are acute and centrally regulated. A dose dependent GnRH-mediated effect of kisspeptin on the testes can cause damages with loss of Sertoli and germ cells. If kisspeptins represent a potential therapeutic tool for reproductive and pubertal disorders, this study alerts us regarding the choice of the doses used.

Kp-54 induced testicular degeneration in adult male rats. The authors show that it is not the continuous administration of kisspeptin that is responsible for the testicular damage, as had previously been published. An acute single high dose of Kp-54 (50 nmol) is sufficient to alter testis histology whereas a low dose of 0.5 nmol kisspeptin has no effect. This effect on the testes was apparent before the desensitization of the gonadotropic axis which came later after kisspeptin administration.

Kisspeptin was initially described as the most powerful secretagogue of GnRH. As shown in the paper of Roseweir et al. (see above), a kisspeptin antagonist blocks GnRH release as well as LH increase in gonadectomized animals. The main effect of kisspeptin is thus the regulation of GnRH release. The results reported by Thompson et al. were unexpected. The authors interpret their results as an acute hyper-stimulation of the HPG axis. However, acute stimulation of the gonadotropic axis by GnRH agonists is frequently used in medicine and such a very rapid effect on the testes is uncommon. The understanding of this side effect of kisspeptin injection on testes needs probably prior understanding of the kisspeptin effect on the pituitary. Testicular degeneration could also be due to an association of central and peripheral mechanisms.

Gamma-aminobutyric acid B receptor mediated inhibition of gonadotropin-releasing hormone neurons is suppressed by kisspeptin-G protein-coupled receptor 54 signaling

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Background: GnRH neurons express both γ -aminobutyric acid (GABA) class of receptors, GABA_A which is a ligand-gated chloride channel, and GABA_B, which is a metabotropic G $\alpha_{i/o}$ -coupled receptor known to activate postsynaptic K⁺ channels or inhibit presynaptic Ca²⁺ channels. Recent studies have shown that Cl currents are activated by GABA in GnRH neurons and that these effects are blocked by the GABA_A receptor antagonist bicuculline. However, the potential role of GABA_B receptor signaling in GnRH neurons remains unknown.

Methods and Results: The authors used whole cell electrophysiological recordings from mouse eGFP-expressing GnRH neurons to show that GnRH neurons were hyperpolarized in a concentration-dependent manner by baclophen, a GABA_B receptor antagonist, through the activation of an inwardly rectifying K⁺ current. The presence of 17 β -estradiol did not have any impact on this activation. However, addition of a selective GABA_B receptor antagonist, CGP52432, abrogated this effect. In a similar manner, the GABA_A receptor antagonist picrotoxin also hyperpolarized GnRH neurons. The authors next assessed whether G-protein-coupled receptor 54 (GPR54) agonist Kisspeptin-10 (Kp10) could alter GABA_B-mediated response and showed that, indeed, baclophen in the presence of submaximal concentrations of Kp10 no longer hyperpolarized GnRH neurons.

Conclusion: Kp10 can desensitize the GABA_B-mediated response, suggesting that the GABA_B receptor may lower GnRH release pulses during 17 β -estradiol negative feedback and that this effect is inhibited by kisspeptin during positive feedback.

The authors show that a majority of GnRH neurons express mRNA of both subunits R1 and R2 of the GABA_B receptor and that baclophen, a selective agonist of the GABA_B receptor, induces a strong hyperpolarization of GnRH neurons. Therefore, GABA may provide a strong inhibitory effect on GnRH neurons to support 17 β -estradiol-mediated negative feedback. Another important result in this study comes from the observation that kisspeptin counters the GABA_B-mediated response through the activation of GPR54, thus providing a better understanding of how GnRH neurons could become excited during the preovulatory phase and how kisspeptins can modulate GnRH pulses.

New genes: a novel unexpected neuropeptide in the central regulation of the gonadotropic axis

TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in the central control of reproduction

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Background: The reactivation of the GnRH pulse at pubertal onset is a fundamental event. This reactivation drives the increase in gonadotropin secretion and then, gonadal steroid secretion and gametogenesis. Several genes are now associated with isolated gonadotropic deficiency, but a few familial cases

still remain unlinked to those genes. In this paper, Topaloglu et al. have performed genome mapping in informative families with isolated gonadotropic deficiency.

Methods: Nine consanguineous Turkish families with normosmic hypogonadotropic hypogonadism (nHH) were subjected to genome-wide SNP analysis after ruling out sequencing mutations in the known hypogonadotropic hypogonadism genes.

Results: A candidate region was found on chromosome 4. Among candidate genes, homozygous loss of function mutations in TACR3 (neurokinin receptor) were identified in 3 families and in TAC3 (neurokinin) in 1 other pedigree. None of the TAC3R mutations were found in 100 ethnically matched controls nor in the 6 other analyzed families or in 50 individuals with nHH from various ethnic groups. The tachykinin neurokinin B (TAC3) is expressed in kisspeptin neurons, namely in the arcuate nucleus, and its receptor (TACR3) is found in rodent GnRH-expressing neurons localized within the median eminence of the hypothalamus.

Conclusion: nHH may be due to inactivating mutations of TAC3 or TAC3R. These findings suggest that the TAC3/TACR3 system is a key positive regulator of the central neuroendocrine control of the reproductive axis.

Hypogonadotropic hypogonadism may or may not be associated with anosmia (respectively nHH or Kallmann syndrome). Human genetics enabled the discovery of some essential genes implicated in the central control of the gonadotropic axis (Kal1, FGFR1, PROK2/PROKR2, GnRHR, KiSS-1/GPR54, etc.). The description of the TAC3/TACR3 inactivation mutation discovered a new gene in the cascade and a new cause of normosmic hypogonadotropic hypogonadism.

The TAC3 mutations were associated with a phenotype of mild learning disability in both affected subjects in this report, but patients with TACR3 mutations were normal. The mechanisms of the gonadotropic axis regulation by neurokinin B remains to be established. This role in rodents has never been suspected because TACR3 invalidated mice are fertile. A probable action at the level of hypothalamic GnRH release is suspected as neurokinin B is expressed in *KiSS-1* neuron axons located in apposition with GnRH neurons expressing both GPR54 and TACR3.

Finally this report opens the way for new pharmacological approaches in the control of reproduction and other hormonally related diseases such as breast and prostate cancer.

Concept revised: involvement of additional genes in the postnatal maturation of the hypothalamus

Developmental profiles of neuroendocrine gene expression in the preoptic area of male rats

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Background: Reproductive function is controlled by GnRH neurons and their steroid-sensitive regulatory inputs. The proper maturation of this system is critical to sexual development at puberty and maintenance of adult reproductive function. Regulation of GnRH release is the main central mechanism regulating reproduction. However, molecular mechanisms underlying this regulation are poorly understood as it is a dynamic process, with sex dimorphism and highly regulated by gonadal hormones. In this study, Walker et al. designed a protocol to analyze differential expression in preoptic area (POA) of 5 genes that form a critical part of the neural circuit controlling reproduction, and they also performed a low-density array.

Methods: A developmental profile of the 5 genes, GnRH, estrogen receptor (ER) α , ER β , androgen receptor (AR) and progesterone receptor (PR), from postnatal day P1 through P60 in the POA was performed in male Sprague-Dawley rats. Serum hormones were measured. A TaqMan low-density array (TLDA) was performed on 48 genes belonging to the estrogen-signaling pathway.

Results: Of the 5 targeted genes, only AR and PR changed robustly (7- and 3- to 4-fold increases, respectively) between P1 and P60. All of the gonadal serum hormones changed markedly: testosterone

decreased from P1 to P30 and then increased to P60; progesterone peaked at P30, and estradiol decreased from P1 to P30 and then stayed low until P60. Using the TLDA, the authors identified several genes that dramatically changed in the POA, particularly G protein-coupled receptor 30, IGF-I, vitamin D receptor, estrogen-related receptor- α , and thyroid receptor- α .

Conclusion: These data demonstrate postnatal variations in genes expressed in the POA, particularly AR and PR. Moreover, the relationships between hormones and their corresponding receptors undergo dynamic changes across postnatal development in the hypothalamus of male rats.

Several groups have tried to characterize transcriptomic changes in the hypothalamus between birth and puberty. The analysis reported in this paper is novel because previous studies tested a single gene or used microarray on the whole hypothalamus to perform the initial screening. In this study, Walker et al. have restricted their analysis to POA and used the TLDA screening technique. TLDA is a PCR method which is very sensitive and specific. They found a small but significant increase in GnRH mRNA through classical quantitative PCR analysis but a small decrease using the TLDA technique. These contradictory results reinforce the concept that translational and posttranslational regulation of GnRH is responsible for the pubertal increase in pulsatile GnRH release at puberty. The large increase in AR suggests that prenatal POA regulation by androgens is not dependant on AR. This result is in line with the concept that masculinization of the POA involves the action of estradiol in early postnatal development. The characterization of PR mRNA increasing in POA is new. This increase is associated with an increase in progesterone levels. The role of progesterone in the postnatal maturation of the hypothalamus needs to be explored. TLDA analysis also revealed a decrease in GPR30 and IGF-1 gene expression. GPR30 encodes a cell membrane estrogen receptor. Its function remains obscure, it probably is involved in negative and positive feedback of estradiol on GnRH neurons. The decrease in IGF-1 was unexpected. This growth factor is better known as an activator of the gonadotropic axis than an inhibitor. However, this decrease may be related to the estradiol decrease observed in this study. The large variation in vitamin D receptor expression with a peak at P45 is new and suggests a role in mechanisms immediately preceding the pubertal initiation. The confirmation of such a scenario in humans is difficult but again analysis of the specific phenotype might be very informative.

New concept: maternal care determines the age of the sexual maturity

Maternal programming of sexual behavior and hypothalamic-pituitary-gonadal function in the female rat

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Background: In many species, maternal effects lead to phenotypic variations in reproductive functions and it is well established that in humans, parental care has a strong influence on the age of puberty, sexual activity during adolescence, and the age at first pregnancy. However, the biological mechanisms involved remain largely unknown, especially during female reproductive development.

Methods and Results: The authors hypothesized that hypothalamic-pituitary-ovarian axis function might be altered by parental care. To assess this hypothesis, they focused on the female offspring of rat mothers with high versus low pup licking and grooming (LG) over the first week postpartum. Compared to those from high LG mothers, adults from low LG mothers showed several differences in sexual compartment, including an increase in the lordosis rating reflecting a higher sexual receptivity, increased LH and progesterone levels at proestrus, an increase in positive feedback of estradiol on LH and GnRH expression in the medial preoptic area, and finally higher levels of estrogen receptor α (ER α) expression in the anteroventral periventricular nucleus. Cross-fostering studies, consisting of removing pups from their biological mother and placing them under the care of mother with the same or different LG phenotype brings clear evidence of a postnatal effect of maternal care but did not rule out an influence of prenatal environment. This is indeed supported by the fact that testosterone levels on embryonic day 20 were higher in fetuses from high LG mothers compared to those from low LG mothers.

Conclusion: This study shows that maternal care in rats has an impact on the hypothalamic-pituitary-ovarian axis development leading to differences in sexual behavior of the female offspring, and that it could result from variations in ER α expression in the anteroventral periventricular nucleus of the hypothalamus.

The age at sexual maturation is highly variable. It is determined by genetic factors as well as environmental factors. The paper of Cameron et al. highlights the consequences of maternal care in the maturation of the function of reproduction. The advanced age of offspring of LG mothers suggests that in adverse conditions, the optimal strategy for the survival of the species is to maximize reproduction by an earlier age at sexual maturity and the number of offspring. As described in the paper of Chang et al. showing the effect on offspring of maternal feeding during pregnancy (see above), the effect of low maternal care on offspring may be interpreted as a genetic trait. In fact, the response to adverse conditions will be similar across generations and therefore transmitted from mothers to daughters. It is well known that adopted children from developed countries are more prone to advanced puberty. The low maternal care provided to these children under an adverse environment may be one explanation.

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