Neuroendocrinology

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New gene

The most obvious candidate gene in gonadotropic deficiency validated at last

Congenital idiopathic hypogonadotropic hypogonadism (IHH) is a condition characterized by a failure to undergo puberty and normal fertility in adulthood. IHH is related to a defect of GnRH secretion or GnRH activity. Genetic defects have now been described in several genes. However, to date, no mutations had been described in the most obvious candidate gene, GnRH itself. Two papers published this year have reported loss of function mutations in few cases of IHH.

Isolated familial hypogonadotropic hypogonadism and a GNRH1 mutation

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Methods and Results: Mutations in GnRH1 which encode for the preprohormone were screened in a population of 310 hypogonadotropic hypogonadism cases. One homozygous frameshift mutation, an insertion of an adenine at nucleotide position 18, was found in 2 siblings from consanguineous parents. Both parents and 1 unaffected sibling were heterozygous for the insertion. Both affected patients have the same phenotype with isolated gonadotropic deficiency without anosmia. In transfected cells this insertion results in an aberrant peptide lacking the conserved GnRH decapeptide. The phenotype was reversed by pulsatile GnRH administration.

Conclusion: This study confirms the pivotal role of GnRH in human reproduction.

GNRH1 mutations in patients with idiopathic hypogonadotropic hypogonadism

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Methods and Results: GnRH1 mutations were screened in 290 hypogonadotropic hypogonadism cases. One homozygous frameshift mutation leading to the synthesis of a peptide truncated at the C-terminal end of the mature peptide. This frameshift mutation was not seen in 192 controls. The mutation was found in a male with micropenis at birth, bilateral cryptorchidism and absent puberty. Four heterozygous variants were also described in 4 patients and were not seen in controls. One non-synonymous missense mutation in the eighth amino acid of the GnRH decapeptide. One nonsense mutation that causes premature termination within the GnRH-associated peptide and 2 sequence variants that cause non-synonymous amino-acid substitutions in the signal peptide and in the GnRH-associated peptide.

Conclusion: GnRH1 mutation is a new genetic cause of normomics isolated hypogonadotropic hypogonadism.

Screening for GnRH1 mutation was the first hypothesis tested to explain congenital IHH without anosmia. In the early 1990s, large numbers of patients were tested without success. A polymorphism

which did not fit with the hypothesis of a mendelian genetic disease with recessive transmission was reported and GnRH1 was therefore rejected. In 2009, two papers appeared at the same time and both reported a very low frequency of GnRH1 mutation in 310 or 290 IHH cases. The phenotype reported by both groups is very similar. In all cases, there was no anosmia, the gonadotropic deficiency was isolated and other endocrine functions of the anterior pituitary were normal. Both patients had blunted response to a single GnRH administration which represents an interesting finding. It confirms that the GnRH test is not appropriate to discriminate hypothalamic or pituitary defects in IHH. This point had already been described 10 years ago when the first mutation of the GnRH receptor was described in patients with low testosterone and estradiol levels, normal LH and FSH levels and adequate response to GnRH administration [1]. After 13 days of pulsatile GnRH administration, LH pulses were detected in 1 GnRH-mutated female and correlated to the GnRH pulses. A similar response was observed in patients with partial gonadotropic deficiency due to GnRH receptor mutation.

The pivotal role of GnRH in the regulation of the gonadotropic axis has been known for more than 20 years. These papers confirm that the physiological function of GnRH1 in humans is restricted to the regulation of the gonadotropic axis. The very low frequency of GnRH1 mutations indicates that it should not be the first gene to be screened in patients with IHH.

New mechanism **Role of estrogen in brain sexual dimorphism**

Estrogen masculinizes neural pathways and sex-specific behaviors

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Background: It has been clearly demonstrated that male sexual behaviors rely on a specific neural network development that requires the sex hormones testosterone and estrogen. However, how both hormonal pathways interact to govern this sexually dimorphic neural circuit remains unknown. Testosterone acts via the androgen receptor (AR) and is also metabolized in the brain into estrogens by aromatase. The authors therefore speculated that aromatase expression could be the key link between the two hormonal pathways.

Methods and Results: In order to determine whether estrogens or testosterone participate in aromatase expression regulation, they designed a knock-in mouse model in which aromatase-expressing neurons specifically express the two enzymatic reporters, β -galactosidase and placental alkaline phosphatase. The authors show a highly sexually dimorphic distribution of aromatase-expressing neurons in terms of number and projections in the bed nucleus of the stria terminalis and the posterodorsal component of the medial amygdale. Male mice lacking a functional AR still display this sexually dimorphic trait. However, neonatal administration of estrogen or testosterone in female mice induced a pattern of brain aromatase expression similar to wild-type males. The analysis of specific apoptosis markers expression in aromatase neurons of bed nucleus of the stria terminalis and medial amygdale shows a higher level of apoptotic cells in young females relative to their male counterparts. Female mice neonatally exposed to estrogen display an increase of territorial urine marking and aggression as well as a reduced sexual receptivity to males mounting and intromission attempts.

Conclusion: This study shows that the sexually dimorphic expression of aromatase is not controlled by AR. Since estrogen is barely undetectable in the male circulation, a local conversion of testosterone into estrogen by aromatase in the brain is likely to explain how estrogen determines the male-specific differentiation of aromatase neurons, probably by promoting cell survival of aromatase-expressing neurons.

The authors have designed a knock-in mouse line expressing reporter enzymes in aromatase-expressing neurons. This allowed them to highlight a sexual dimorphism of the aromatase-expressing neural network in the bed nucleus of the stria terminalis and medial amygdale, which govern the acquisition of typical male sexual behaviors, such as aggressiveness and territorial marking. In female neo-

nates, ovaries are inactive whereas males display a surge in testosterone that is absolutely required for proper male sexual behavior acquisition. Here the authors bring evidence that this neonatal surge allows local testosterone aromatization into estrogen, the latter protecting the aromatase-expressing neurons from entering apoptosis.

The major role of estrogen, testosterone and aromatase in the acquisition of male sexual behavior has been known for over 20 years. However, the underlying mechanisms have always remained unclear, especially the balanced roles between testosterone and estrogen. This work therefore brings precious insight into these mechanisms. It also raises numerous questions on how local conversion of testosterone into estrogen promotes aromatase neurons survival in the BNST and MeA, and whether other sexually dimorphic populations of neurons are determined through such a mechanism.

Conditional inactivation of androgen receptor gene in the nervous system: effects on male behavioral and neuroendocrine responses

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J Neurosci 2009;29:4461–4470

Background: In the developing male, testosterone (T) plays a crucial role in the establishment of male-specific neuronal network, governing typical male sexual behavior. T signaling is mediated either directly via the androgen receptor (AR) or indirectly via the estrogen receptor (ER) after T aromatization into estradiol. Because T and AR also exert major peripheral effect on male gonadal differentiation and several other physiological functions, the balance between central and peripheral influence of T on the determinism of male behavior and neuroendocrine control of reproduction is unclear.

Methods and Results: In order to distinguish between the central and peripheral roles of AR, the authors used the Cre-loxP technology to generate mutant mice specifically lacking AR expression in the nervous system only (AR^{NesCre}), thereby maintaining functional AR signaling in the peripheral tissues. Mutant males were fertile and phenotypic analyses showed that central T has little influence on the urogenital tract development. However, T and LH secretions were twofold higher than in wild-type animals with no changes in FSH levels. Mutant mice were as receptive to female pheromones as wild-type mice in terms of neuronal activity and olfaction, but showed severely impaired masculine behaviors, such as erectile activity, mounting and aggressiveness. Finally, the authors demonstrate the role of central AR in the somatotrophic axis, as mutant mice showed reduced IGF-I levels and growth retardation.

Conclusion: These results show that central AR is involved in male-specific acquisition of sexual and aggressive behaviors, and participates in the regulation of the somatotrophic axis.

In this study, the authors designed a powerful genetic model to explore the specific role of central AR in mice. Loss of central AR did not abrogate fertility, although the authors report a high proportion of hypofertile males. Because LH secretion is increased twofold in AR^{NesCre} mice, the data strongly suggest the existence of a major T-dependent negative feedback on GnRH release as well as a pituitary positive feedback on LH secretion, confirming previous indirect evidence of T feedback provided by the study of ER α knockout male mice [2]. The authors did not detect AR expression in GnRH neurons, suggesting that interneurons such as kisspeptin neurons, which express AR [3], may convey T signal toward GnRH neurons. Analysis of Kiss1 expression in AR^{NesCre} mice would allow to test this hypothesis.

Male AR^{NesCre} mice display strongly impaired masculine behavior, suggesting that T signaling through AR in the brain plays a crucial role in the establishment of a male-specific neuronal network. However, Juntti et al. [4] recently generated another neuron-specific AR knockout mouse line called AR^{NsDel}. In contrast with AR^{NesCre} mice, male AR^{NsDel} display only moderately impaired male behaviors. Moreover, the authors show that AR expression in the brain at the time of the androgen surge is very weak and is regulated by ERs. Therefore, the authors propose that estrogen metabolized from testosterone during development is responsible for a higher expression of AR in the adult brain of males, where T signals augment the male-pattern behaviors that have been differentiated under the control of estrogen signaling.

Role of estradiol in the dynamic control of tanycyte plasticity mediated by vascular endothelial cells in the median eminence

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Endocrinology 2010;151:1760–1772

Background: During low GnRH outputs, GnRH-secreting axons are enveloped by ependymoglia cells named tanycytes, which prevent GnRH crossing the basal lamina of the brain to reach the pituitary portal circulation. Prior to the preovulatory surge of LH, tanycytes no longer create a physical barrier between GnRH neuron terminal axons, therefore allowing GnRH release into the circulation. The authors previously provided evidence that endothelial cells of the median eminence play a key role in the modulation of neuroglial remodeling through the nitric oxide (NO) pathway, but the molecular mechanism governing tanycyte retraction in response to GnRH output requirements still remain unclear.

Methods and Results: The authors show that tanycytes cocultured with endothelial cells of the median eminence (ECME) present an important retraction under short estradiol exposure. When cultured alone or cocultured with ECME in the presence of a NO synthase-dominant negative form, tanycytes do not retract. Triggering of NO release by L-arginine in estradiol-free cocultures induces tanycytes retracting, suggesting that estradiol triggers tanycytes retraction by inducing a NO release by ECME. Cyclooxygenase (COX)1 and COX2 are known to be involved in NO-induced actin cytoskeleton remodeling of tanycytes. Estradiol upregulates both COX1 and COX2 expression in tanycytes, leading to an increase of prostaglandin E₂ (PGE₂) synthesis. PGE₂ treatment induces an increase of retraction of cultured tanycytes. Ultrastructural analysis of mouse hypothalamic explants of the ME showed that PGE₂ induces an increase of the distance between GnRH nerve terminals and the basal lamina, suggesting a retraction of the surrounding tanycytes. Finally, they show that in vivo inhibition of COX activity in mice impairs the estrous cycle, thereby confirming the important role of COX and PGE₂ in the regulation of the estrous cycle.

Conclusion: The authors show that vascular endothelial cells play a critical role in the neuroendocrine brain plasticity by conveying estradiol signaling toward tanycytes in the ME.

It is now well established that neuronal signaling at the cellular level is not solely dependent on other neuronal inputs, but strongly relies on the microenvironment formed by the surrounding glia and brain capillaries. The events occurring in the median eminence of the hypothalamus during the ovarian cycle are a striking example of the dynamic morphological changes controlling neurosecretory axons. This elegant work brings further insight into the mechanisms on how endothelial cells, tanycytes and neurons coordinate their activity in response to changing outputs, such as estradiol. Here the authors suggest that estradiol induces NO release from endothelial cells of the median eminence, resulting in tanycytes retraction. This retraction could be facilitated by an estradiol-dependent increase of cyclooxygenases COX1 and 2, which metabolize PGE₂, a modulator of tanycyte plasticity. These results add a new level of complexity to the understanding of estradiol-induced rise of GnRH secretion prior to the LH preovulatory surge. The question of how the negative estradiol feedback switches to a positive feedback remains unanswered.

Postnatal development of an estradiol-kisspeptin-positive feedback mechanism implicated in puberty onset

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Endocrinology 2009;150:3214–3220

Background: Over the past years, the kisspeptin neuropeptide encoded by KISS1 has been extensively described as a key part in the activation of the gonadotropic axis at puberty through the modulation of GnRH release. However, the mechanisms underlying the increase of KISS1 expression in the hypothalamus prior to the onset of puberty remain unknown.

Methods and Results: Using immunocytochemistry, the authors first determined the postnatal profile of kisspeptin expression in the rostral periventricular area of the third ventricle, where kisspeptin neurons

innervate GnRH neurons. They show that kisspeptin immunoreactivity in mouse occurs around the postnatal day (P)15 and reaches its maximal level at P30, which corresponds to the onset of puberty. Female pups were then ovariectomized, which resulted in a very strong reduction in kisspeptin immunoreactivity at P30 and P60. Treatment with exogenous estrogen restores kisspeptin wild-type profile of expression. Furthermore, mice lacking a functional aromatase display a total absence of kisspeptin immunoreactivity in the periventricular area of the third ventricle, and a decrease in the arcuate nucleus.

Conclusion: These results show that ovarian estrogen is required for the postnatal increase of kisspeptin expression in periventricular area of the third ventricle neurons during the prepubertal period.

In this study, the authors bring evidence for an estrogen-driven positive regulation of kisspeptin expression in the hypothalamic region periventricular area of the third ventricle, occurring around P15 in mice. As the kisspeptin/KISS1R system is critical for normal puberty onset, these results account for a positive feedback loop model where estrogen-induced increase of kisspeptin activates GnRH neurons resulting in a rise of gonadotropins, which in turn stimulate gonadal steroids and so on, ultimately leading to the activation of the gonadotropic axis at puberty.

Kisspeptin expression was not detected before P15, suggesting that the early activation of GnRH neurons soon after birth does not depend on kisspeptin, but rather on other neurotransmitters signaling, such as GABA and glutamate. Once ovarian function produces sufficient estrogen level, kisspeptin in periventricular area of the third ventricle facilitates GnRH impulse generator. Whether estrogen acts directly on kisspeptin neurons remains to be defined, but it would be very interesting to compare the mechanisms of prepubertal upregulation of kisspeptin in the periventricular area of the third ventricle with the upregulation occurring in the AVPV in adult females.

Concepts revised **Characterization of kisspeptin neurons**

Forebrain projections of arcuate neurokinin B neurons demonstrated by anterograde tract tracing and monosodium glutamate lesions in the rat

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Neuroscience 2010;166:680–697

Background: Current studies indicate that neurokinin-3 (NK3) receptor and kisspeptin receptor signaling play a key role in the regulation of the gonadotropic axis activity in humans. Some neurons residing in the rat arcuate nucleus (ARC) express neurokinin B (NKB), kisspeptin, dynorphin, NK3 receptor and estrogen receptor. However, how this neuronal network regulates the gonadotropic axis is unknown.

Methods and Results: The projections of these neurons in the ARC were studied using NKB immunohistochemistry as a marker. First, arcuate nucleus was ablated by neonatal injection of monosodium glutamate. Second, neurokinin B fibers were anterogradely labeled using biotinylated dextran amine (BDA) injection into the arcuate nucleus and NKB/BDA immunoreactive axons were labeled. Four major projections pathways are described: (1) local and bilateral projections in the arcuate nucleus, (2) projections into the median eminence, (3) projections toward the periventricular zone, and (4), projections to the lateral and ventral hypothalamic tract and the medial forebrain bundle.

Conclusion: The diverse projections of arcuate NKB neurons provide evidence that NKB/kisspeptin/dynorphin neurons could integrate the reproductive axis with multiple homeostatic, behavior and neuroendocrine processes. This study reveals a bilateral interconnected network of sex-steroid-responsive neurons in the arcuate nucleus of the rat that project GnRH terminals in the median eminence. This circuitry provides a mechanism to modulate the pulsatile secretion of GnRH.

Neurokinin B and dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat

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J Neurosci 2010;30:3124–3132

Background: The pulsatile release of GnRH secretion is essential to drive pulsatile gonadotropin secretion and normal reproduction in mammals. Kisspeptin neurons serve as the central pacemaker that drives GnRH secretion.

Methods and Results: Using immunohistochemistry, electrophysiological techniques and central injection of dynorphin (DynA), neurokinin B (NKB) or κ -opiate receptor (KOR) antagonist, the authors have characterized kisspeptin neurons in the goat arcuate nucleus (ARC) and evaluated their role in GnRH secretion regulation. Immunohistochemistry confirmed that kisspeptin, dynorphin and neurokinin B are coexpressed in the same population of neurons. Electrophysiological techniques showed that bursts of multiple-unit activity (MUA) occurring in the medial basal hypothalamus of ovariectomized animals are associated with discrete pulses of LH. The frequency of these bursts is reduced by gonadal steroids. Central injection of dynorphin inhibits MUA volleys and pulsatile LH secretion, whereas neurokinin B induces multiple-unit activity volleys.

Conclusion: Kisspeptin neurons in ARC regulate GnRH secretion, and dynorphin and neurokinin B seem to be involved in the process leading to the rhythmic discharge of kisspeptin in goat.

Tac3 encodes neurokinin B which is the ligand of the neurokinin B receptor encoded by *Tacr3*. Mutations in *Tac3* and *Tacr3* have been recently associated with gonadotropic axis deficiency [Yearbook 2009, pp 9–10]. In the human infundibular nucleus, a group of sex steroid-responsive neurons expresses neurokinin B, kisspeptin and dynorphin, a similar group of neurons were described in monkeys. Although *Tacr3*-invalidated adult mice were fertile, a group of kisspeptin neurons express neurokinin B, dynorphin, and the estrogen receptor- α which suggest that this group of neurons also participates in the hypothalamic regulation of estrogen-negative feedback on the mouse gonadotropic axis. It is therefore fundamental to characterize the neuronal network involved in this regulation and its physiological role in the gonadotropic axis homeostasis.

The first article provides compelling evidence that the majority of the neurokinin B-immunoreactive axons within the median eminence in rat originate from the arcuate nucleus and modulate GnRH secretion through the neurokinin-3 receptor via non-synaptic transmission. The importance of the neurokinin B receptor is underscored by its expression both in arcuate neurokinin B neurons and GnRH terminals in the median eminence. The multiple projections of these neurons suggest that it could also participate in the regulation of other homeostatic, behavioral and neuroendocrine circuits.

In the second article, the authors provide evidence that dynorphin and neurokinin B indeed participate in the regulation of GnRH pulsatile secretion in goat. This co-expression of the three peptides in ARC neurons is thus a conserved mechanism observed in all mammals studied to date. This study also suggests a physiological function for dynorphin and neurokinin B. Kisspeptin neurons in the ARC drive pulsatile GnRH and LH secretion. After central administration of neurokinin B, dynorphin, or KOR antagonist, electrophysiological techniques show that the multiple unit activity in close proximity of kisspeptin neurons are influenced by neurokinin B and dynorphin A. The arcuate nucleus KiSS/NKB/Dyn neurons network probably acts as a GnRH pulse generator: NKB/NK3R signalling stimulates and the Dyn/Kor signalling inhibits the activation of KiSS/NKB/Dyn neurons. These results are consistent with a model in which the pulsatile secretion of GnRH is related to a rhythmic oscillation activity in kisspeptin neurons leading to rhythmic secretion of kisspeptin. They do not preclude a similar activating-inhibiting loop on the GnRH neurons. In conclusion, these two papers confirm the essential role of neurokinin and dynorphin in the regulation of arcuate nucleus neurons expressing kisspeptin.

New hope Sim1 as a new target in obesity

Postnatal Sim1 deficiency causes hyperphagic obesity and reduced mc4r and oxytocin expression

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Background: Mutations in single-minded 1 (*Sim1*), which encodes a transcription factor, are associated with a monogenic obesity in humans and mice. The physiological postnatal functions of this protein have not been well established.

Methods and Results: The authors designed a mouse line with a conditional postnatal CNS deletion of *Sim1* by crossing α -calcium/calmodulin-dependent protein kinase II-Cre mice with mice carrying a floxed *Sim1* allele. This conditional postnatal deletion was sufficient to cause hyperphagic obesity in *Sim1* heterozygotes. Using stereological cell counting, the authors showed no hypocellularity in the paraventricular nucleus of the mutant mice hypothalamus and normal neuronal PVN projections. However, *MC4R* and oxytocin mRNAs were significantly decreased in this area in both conditional *Sim1* homozygotes and germ line *Sim1* heterozygotes.

Conclusion: The hyperphagic *Sim1*-deficient mouse becomes obese because of feeding dysregulation due to changes in the leptin-melanocortin-oxytocin pathway.

Single-minded 1 (*Sim1*) deletions seem to be one of the few causes of monogenic isolated (non-syndromic) obesity but the mechanism involved in the phenotype is unknown. *Sim1* null mice die perinatally and lack anterior periventricular paraventricular and supraoptic hypothalamic nuclei. Phenotypic analyses performed in heterozygote animals did not allow to conclude whether *Sim1* is only involved in hypothalamic development or also participates in postnatal physiology of the hypothalamus. This study demonstrates a role for *Sim1* in food intake regulation after birth by modulating the leptin-melanocortin-oxytocin pathway. The decrease in oxytocin PVN expression after *Sim1* invalidation suggests a possible role of oxytocin deficiency in the hyperphagia observed in this mouse. To support this hypothesis, the phenotype was rescued by oxytocin treatment. However, decreased expression of *mc4r* could also explain hyperphagia. Additional studies are needed to further identify which of *Sim1* targets are indeed regulating food intake. This study also suggests that *Sim1* and its potential target genes act postnatally in paraventricular hypothalamic nucleus and could be potential therapeutic targets for appetite suppression in obese individuals.

New mechanism Molecular Interface between food intake and reproduction

Melanin-concentrating hormone directly inhibits GnRH neurons and blocks kisspeptin activation, linking energy balance to reproduction

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Background: The link between energy balance and reproduction is critical. In negative energy balance, reproductive processes need to be aborted but molecular mechanisms mediating this link remain unclear. Fasting and food restriction up-regulate the hypothalamic melanin-concentrating hormone (MCH) system that promotes feeding and decreases energy expenditure; MCH knockout mice are lean and have a higher metabolism but remain fertile. MCH also regulates other hypothalamic related function such as

sleep, drug abuse, behavior and mood. Kisspeptin is the major regulator of GnRH secretion and therefore the central regulator of the gonadotropic axis. Two subpopulations of GnRH neurons have been described, one of which expresses the vesicular glutamate transporter 2 (vGluT2).

Methods and Results: In this study, Wu et al. have tested the hypothesis that the link between feeding regulation and reproduction is mediated via a direct postsynaptic effect on vGluT2-GnRH neurons activated by kisspeptin. Using patch-clamp recording in multiple lines of transgenic mice, they demonstrated a strong inhibitory effect of MCH on vGluT2-GnRH neurons. MCH has no effect on kisspeptin-insensitive GnRH, VGlut2, cholinergic or GABAergic neurons located in the same hypothalamic nucleus. MCH blocks the excitatory effect of kisspeptin on vGluT2-GnRH neurons. This inhibitory effect of MCH is mediated via a direct postsynaptic BA^{2+} -sensitive K^+ channel mechanism involving the MCHR1 receptor. MCH fibers are in close proximity to VGlut2-GFP and GnRH-GFP neurons.

Conclusion: Considering the role of MCH in regulating energy balance and of kisspeptin in regulating fertility and pubertal onset, MCH may provide a critical link between energy balance and reproduction.

It is now clear that kisspeptin is the major regulator of the GnRH secretion and therefore it is involved in the timing of the pubertal onset and in the occurrence of the LH surge leading to ovulation. Normal energy balance is critical for normal fertility which occurs only when metabolic fuel is available. Peptides conveying metabolic fuel to brain act at different levels of the gonadotropic axis. Some of them, such as insulin, act directly on GnRH neurons, others such as leptin, target kisspeptin neurons. This study reports a combined effect of melanin-concentrating hormone on these two neuroendocrine targets by showing that melanin-concentrating hormone can inhibit the excitatory effect of kisspeptin on vGluT2-GnRH neurons. The most intriguing result reported here is the capacity of melanin-concentrating hormone to specifically target kisspeptin-activated GnRH neurons and not GnRH neurons refractory to kisspeptin activation. This result provides an additional evidence of the presence of at least two GnRH neuron populations in the normal hypothalamus. This inhibitory effect of melanin-concentrating hormone was observed in prepubertal as well as pubertal mice in both sexes. The normal fertility in melanin-concentrating hormone knockout indicates that melanin-concentrating hormone effect on the gonadotropic axis is mainly required to curtail reproductive activity in conditions such as food deprivation. The next step of this interesting model would be to demonstrate that the inhibitory post-synaptic effect of melanin-concentrating hormone revealed by patch-clamp is associated with a decrease of kisspeptin-induced GnRH secretion.

New mechanism Leptin and GnRH secretion

Leptin indirectly regulates gonadotropin-releasing hormone neuronal function

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Endocrinology 2009;150:2805–1812

Background: Environmental regulation of GnRH neuronal activity determines the reproductive status of the individual. Leptin is one of the hormones communicating information about metabolic status to the hypothalamic GnRH neuronal system.

Methods and Results: To determine whether leptin acts directly on GnRH neurons, the authors used three complementary approaches. First, pSTAT3 immunopositive GnRH neurons were counted after intraventricular injection of leptin in males and females rats. Fertility was assessed in males and females with conditional leptin receptor knockout mice from all forebrain neurons or GnRH neurons only. Leptin receptor deletion in forebrain neurons prevented the onset of puberty resulting in infertility and blunted estradiol-induced LH surge. Leptin receptor deletion in GnRH neurons did not impair fertility. Finally, single cell-nested PCR in living brain slices showed that GnRH neurons do not express leptin receptor

mRNA. Intracerebroventricular leptin treatment induced pSTAT3 expression within the AVPV but not in GnRH neurons.

Conclusion: GnRH neurons are not direct targets for leptin in rodents.

Normal fertility was observed in GnRH neuron conditional leptin receptor deletion. Single cell-nested PCR confirmed the absence of leptin receptor mRNA in GnRH neurons and pSTAT3 immunohistochemistry showed no co-localization with GnRH labelling. The possibility that leptin receptor could stimulate other signalling pathways than pSTAT3 cannot be excluded; however, pSTAT3 neuronal knockout is the only component of the leptin signalling pathway that results in infertility and recapitulates the leptin receptor KO phenotype. This study definitively confirms that leptin acts on the GnRH system via afferent interneurons. Kisspeptin neurons are probably one of the candidate interneurons to be tested.

New hormone

A new neuropeptide involved in the regulation of food intake

Neuropeptide W: an anorectic peptide regulated by leptin and metabolic state

Date Y, Mondal MS, Kageyama H, Ghamari-Langroudi M, Takenoya F, Yamaguchi H, Shimomura Y, Mori M, Murakami N, Shioda S, Cone RD, Nakazato M

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Endocrinology 2010;151:2200–2210

Background: Neuropeptide W (NPW) is an anorectic peptide produced in the brain but its interactions with other peptides that regulate feeding are unknown.

Methods and Results: NPW is present in several nuclei of the hypothalamus including the paraventricular, ventromedial, lateral and arcuate nuclei. NPW expression was significantly up-regulated in leptin-deficient and leptin-receptor-deficient mice. In leptin-deficient mice, leptin replacement rescued NPW hypothalamic levels. Leptin regulation of NPW expression is controlled by phosphorylation of STAT3 which induces SOCS3 in NPW-expressing neurons. The authors also report that NPW reduces feeding potentially via the melanocortin-4-receptor signaling pathway, activates POMC and inhibits NPY neurons in mice expressing promoter-driven green fluorescent protein.

Conclusion: NPW plays an important role regulating feeding under the conditions of leptin insufficiency.

Previous reports have shown that NPW is expressed in different hypothalamic nuclei known as essential areas in the regulation of energy metabolism and feeding behavior. Here, it is shown that NPW expression increases under low leptin levels, which was a surprising result. Usually, anorectic peptides are up-regulated whereas orexigenic peptides are down-regulated by leptin. This control of leptin on NPW seems to be direct via activation of the leptin receptor in NPW-expressing neurons. The exact intracellular signaling pathways governing this control remain unclear. The authors studied the effect of leptin on NPW neurons in primary cells culture from adult rats and it will be necessary to confirm this effect using an in vivo approach. These results add a new complexity in the hypothalamic network regulating feeding behavior in low leptin condition. NPW seems to be a new example of a peptide having a predominant role in response to specific circumstances. It is necessary to study whether the role of NPW under normal conditions is similar.

New mechanism

From the islet to the brain and back

Influence of insulin in the ventromedial hypothalamus on pancreatic glucagon secretion in vivo

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Background: The hypothesis that insulin regulates pancreatic α -cell glucagon secretion by a central effect on the hypothalamus has not been well investigated.

Methods and Results: To study whether insulin acts within the ventromedial hypothalamus (VMH) to modulate the secretion of glucoregulatory hormones, the authors compared the effects of VMH exposure to insulin, anti-insulin antibody, insulin receptor antagonist, a combination of anti-insulin antibody plus GABA receptor agonist muscimol prior to a hypoglycemic clamp or under baseline conditions. Pancreatic glucagon response to central insulin was 4- to 5-fold lower than in the absence of systemic hyperinsulinemia with phloridzin-induced hypoglycemia. VMH insulin action directly suppressed glucagon response to insulin-induced hypoglycemia independently of intra-islet insulin or input from circulating catecholamines. VMH insulin microinjection suppressed glucagon response to phloridzin-induced hypoglycemia. Local blockade of VMH insulin action increased basal glucagon levels, an effect completely abolished by a simultaneous injection of γ -aminobutyric acid A receptor agonist, muscimol.

Conclusion: Insulin inhibits α -cell glucagon secretion both at the level of the pancreas and of the VMH.

Glucose homeostasis is regulated by insulin and glucagon with a subtle interplay between these two hormones and it is known that insulin exerts a paracrine effect in the islet on glucagon. Here, it is shown that insulin also acts centrally to regulate pancreatic glucagon secretion under fasting and mild hypoglycemic conditions. The authors cannot exclude the possibility that VMH surrounding regions also contribute to the changes observed in glucagon secretion because the cerebral injection is difficult to realize. This study provides evidence for a role of insulin signalling in the regulation of α -cell function in vivo. The authors suggest that the primary site for insulin-mediated glucagon regulation is the islet but highlight a secondary role for central mechanisms. An insulin central effect appears to occur under hypoglycemia and a basal condition and might contribute to glucose homeostasis disorders. The loss of glucagon response to hypoglycemia in type 1 diabetic patients might result in part from the simultaneous increase in insulin levels both in the VMH and locally in the islet caused by exogenous insulin administration. Altogether, these data point to the central importance of the hypothalamus in the regulation of metabolic fluxes and their hormonal control.

New mechanism

A central clock preventing enuresis

Central clock excites vasopressin neurons by waking osmosensory afferents during late sleep

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Background: In addition to its major function in the endocrine axis regulation, the hypothalamus is a central regulator of many fundamental behaviors like temperature regulation, food intake and circadian rhythms. Integrated regulations between circadian rhythms and endocrine homeostasis have been

largely described. However, the molecular mechanisms by which the suprachiasmatic nucleus (SCN) regulates hormonal secretion remain unknown.

Methods and Results: Clock neurons of the SCN modulate osmosensory synapses onto vasopressin neurons to facilitate osmoregulated vasopressin release during sleep. This release is facilitated at the end of the sleep cycle to prevent dehydration and enuresis. Previous work has revealed that clock neurons have low firing rates during the late sleep period. The authors found that excitation of rat vasopressin neurons by osmosensory afferents is facilitated during the late sleep period, suggesting a possible regulation of magnocellular neurosecretory cells by SCN neurons.

Conclusion: Clock neurons mediate an activity-dependent presynaptic silencing of osmosensory afferent synapses onto vasopressin neurons. Osmoregulatory gain is enhanced by removal of this effect during late sleep.

Several hormones are produced with a circadian timing and vasopressin is one of them. This biological rhythm over 24 h is fundamental for a normal endocrine homeostasis. Biological rhythms are under the control of the suprachiasmatic nucleus located in the hypothalamus. Neurons of this nucleus exhibit daily rhythmic activities that are regulated by cell autonomous molecular feedback loops involving clock genes. The circadian rhythm is adjusted to 24 h by external clues like daylight. The molecular mechanisms linking suprachiasmatic nucleus circadian activity and daily hormonal rhythms are poorly understood. In this paper, a molecular mechanism to explain the circadian regulation of the homeostatic circuit involved in osmotic balance is proposed. A complex biochemical interactions between osmosensory neurons located in the organum vasculosum lamina terminalis, suprachiasmatic nucleus neurons and vasopressin neurosecretory cells located in the supraoptic nucleus is shown. The experimental approach used by the authors is interesting as they were able to stimulate organum vasculosum laminae terminalis neurons with a hyperosmotic stimulus with or without electrical stimulation of the suprachiasmatic nucleus and then recorded action potentials at distance in magnocellular neurosecretory cells. Their results indicate that the increased release of vasopressin during the end of the sleep cycle is mainly regulated by the release of an inhibitory effect of suprachiasmatic nucleus neurons on osmosensory neurons. The characterization of the biochemical mechanism of this inhibitory effect will be the next step of this study. This work opens the way to understand how suprachiasmatic nucleus neurons can regulate circadian rhythms of different endocrine axes. A better understanding of these molecular mechanisms may also be important for testing new hypothesis and new treatment for nocturnal enuresis.

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