Neuroendocrinology

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The literature on neuroendocrinology is highly diverse. Being scientists involved in endocrinology research, we naturally focused our attention on the central regulation of the endocrine axis. We have therefore selected papers proposing new concepts or new hypothalamic networks to explain these regulations. However, additional papers selected this year show that the hypothalamus cannot be restricted to its function in neuroendocrine regulations. Three points emerge from this selection: (1) It is well known that hypothalamic peptides that have a peripheral role, like oxytocin, may also have central effects. The improvement of in-vivo models of cellular activity has helped to characterize this concept. (2) Autophagy in peripheral tissues contributes to energy homeostasis. Autophagy in specific hypothalamic neurons also plays an important role in the neuroendocrine regulation of metabolism. (3) The role of the hypothalamus in the regulation of arterial tone. This function is highlighted by a paper showing the relation between dysfunctional proopiomelanocortin neurons and the occurrence of obesity-related hypertension. Such a role of hypothalamic neurons in obesity-related hypertension was suspected but not previously demonstrated.

New mechanism

Agouti-related protein and fertility in *Lep^{ob/ob}* mice

Ablation of neurons expressing agouti-related protein, but not melanin-concentrating hormone, in leptin-deficient mice restores metabolic functions and fertility

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Proc Natl Acad Sci USA 2012;109:3155–3160

Background: Nonsense mutation in the leptin gene in mice (*Lep^{ob/ob}* mice) induced obesity, hyperphagia, and infertility. A complex neuronal circuitry seems to be involved in this phenotype.

Methods: In *Lep^{ob/ob}* mice treated with diphtheria toxin (DT), agouti-related protein (AgRP)- or melanin-concentrating hormone (MCH) neurons were selectively ablated due to specific expression of the human DT receptor in either AgRP or MCH neurons.

Results: Ablation of MCH neurons had no effect on food intake, body weight, fertility, but improved glucose tolerance in *Lep^{ob/ob}* mice. Ablation of AgRP neurons in lean or severely obese *Lep^{ob/ob}* mice induced a severe anorexia leading to moribund animals. However, moderately obese *Lep^{ob/ob}* mice survived AgRP neuron ablation and became fertile.

Conclusion: AgRP neurons play a critical role in obesity and infertility of *Lep^{ob/ob}* mice, whereas MCH neurons have only a minor effect.

Loss of leptin or its receptor leads to obesity, diabetes and infertility in humans and rodents. A complex neuronal circuit, including hypothalamic neurons expressing agouti-related protein (AgRP) and melanin-concentrating hormone (MCH), is involved in the body weight regulation and leptin signaling. Ablation of MCH-expressing neurons has a limited effect on metabolic functions in *Lep^{ob/ob}* mice. However, ablation of AgRP neurons induces a severe anorexia in *Lep^{ob/ob}* mice, suggesting that this anorexigenic effect is independent of MCH signaling. Anorexia induced by AgRP ablation is critical in lean and severely obese *Lep^{ob/ob}* mice, while moderately obese *Lep^{ob/ob}* mice survive and become fertile. It is difficult to determine if this differential effect in mice is modified by body weight or age, because body weight increased with the age of the animals (lean, moderate and severely obese mice).

were respectively 6, 8 and 10 weeks old). In older *Lep^{ob/ob}* mice, anorexia is accompanied by a severe decrease in body temperature linked to a decreased output of the sympathetic nervous system. Lethal anorexia induced by AgRP neuron ablation results from the loss of GABA signaling leading to a neuronal hyperactivity, which is compensated in moderate obese mice by an unknown mechanism. Restored fertility in *Lep^{ob/ob}* mice has already been observed in mice lacking NPY. AgRP neuron ablation does not affect all NPY neurons, indicating that restored fertility in *Lep^{ob/ob}* mice with ablation of AgRP neurons is not attributed to an NPY effect. This study highlights the critical role of AgRP neurons in metabolic function and fertility.

Concept revised Kisspeptin release is developmentally regulated

Developmental increase in kisspeptin-54 release in vivo is independent of the pubertal increase in estradiol in female rhesus monkeys (*Macaca mulatta*)

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Endocrinology 2012;153:1887–1897

Background: The pulsatile release of kisspeptins, a potent stimulator of GnRH neurosecretion, is increased during pubertal maturation. The aim of this study was to determine if a developmental regulation of kisspeptin release exists in monkey.

Methods and Results: The pattern of release of Kp54 was measured in vivo in prepubertal and pubertal female monkeys. A developmental increase in mean Kp54 release, pulse frequency and pulse amplitude occurred between prepubertal and pubertal monkeys. Ovariectomy and estrogen administration had no effect on Kp54 in prepubertal animals. However, in pubertal animals, ovariectomy increased mean Kp54 release and pulse amplitude, an effect that was reversed by estrogen.

Conclusion: The pubertal increase of kisspeptin release occurs independently of circulating estrogen level. Kisspeptin release at the pubertal onset is unlikely to be responsible for the puberty onset, but seems to contribute later on to further increase the GnRH release during the progression of puberty.

Puberty is associated with an increased GnRH and kisspeptin pulsatile release. The developmental regulation of GnRH secretion is well understood, but few data are available concerning kisspeptin release. The present in-vivo study highlights a developmental regulation of kisspeptin release which coincides well with the developmental regulation of GnRH release. This strongly supports the role of kisspeptins in the regulation of pulsatile GnRH release during puberty, but also during the night. As for GnRH secretion, kisspeptin release in prepubertal monkeys does not depend on circulating estrogen level. This underscores differences between species. In fact, in prepubertal rodent, kisspeptin and GnRH signaling are regulated by estrogen. The fact that kisspeptin and GnRH release are not regulated by estrogen in prepubertal monkey is in accordance with the existence of a negative neuronal input by GABA on kisspeptin neurons. Altogether, it appears that in female monkey, kisspeptins do not play a critical role in triggering puberty onset, but contribute to the increase of pulsatile GnRH release during pubertal onset, illustrating the complex and species-specific mechanisms of pubertal onset.

Concept revised

The physiological link between kisspeptin and GABA

Tonic control of kisspeptin release in prepubertal monkeys: implications to the mechanism of puberty onset

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Endocrinology 2012;153:3331–3336

Background: Reduction in γ -aminobutyric acid (GABA) inhibition is critical for the pubertal onset by increasing GnRH release. Kisspeptin release in the medial basal hypothalamus is low in prepubertal female monkeys. The authors hypothesized that the low levels of kisspeptin release in prepubertal monkey is due to the tonic GABA inhibition.

Methods: Kisspeptin release in the medial basal hypothalamus was studied by microdialysis in prepubertal and pubertal monkeys treated with bicuculline, a GABA(A) receptor antagonist.

Results: Infusion of bicuculline in prepubertal monkey induced a rapid effect on kisspeptin release. No effect was observed in midpubertal or pubertal monkeys. In a second series of experiments, the authors showed that the bicuculline-induced GnRH release was suppressed by a kisspeptin receptor antagonist.

Conclusion: The low kisspeptin release in the medial basal hypothalamus of prepubertal monkeys is due to a GABA inhibition on kisspeptin neurons.

The balance between inhibitory (GABA) and excitatory (glutamate) neurotransmitters is one mechanism that suppresses gonadotropic axis activity in prepubertal animals and allows activity at pubertal onset. Before pubertal onset, GABA was demonstrated to block GnRH release. At pubertal onset, this GABA inhibition is reduced, leading to an increase of GnRH release and then pubertal onset. This study proposes that the inhibitory effect of GABA on GnRH release is mediated by interneurons such as kisspeptin neurons. Again, this work shows that kisspeptin neurons are the main hypothalamic neurons where regulators of the gonadotrope axis converge. The difference between prepubertal and midpubertal monkeys may be explained by the reduced GABA release between these two stages. Several other regulators of GnRH release have a role in the juvenile period and in pubertal onset. Several of them act through kisspeptin neurons in association or not with GABA. This study adds an additional complexity in the network regulating pubertal onset.

New mechanism

GnIH is a switch between feeding and reproduction

Gonadotropin-inhibitory hormone is a hypothalamic peptide that provides a molecular switch between reproduction and feeding

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Neuroendocrinology 2012;95:305–316

Background: Gonadotropin-inhibitory hormone-3 (GnIH-3) is a hypothalamic peptide that plays a role in the regulation of reproduction and feeding in mammals. However, reciprocal control of reproduction and feeding behavior is not known.

Methods and Results: In situ hybridization in ewes showed that GnIH-3 expression was low during the follicular phase, suggesting that it constitutes a permissive condition for the preovulatory LH surge. Infusion of GnIH-3 in different mammalian species (ewes, mouse, rat, monkey) had no effect on sexual

behavior, but increased food intake without modifying energy expenditure. Immunohistochemistry showed that GnIH-3 increased Fos expression in orexigenic neurons and also in anorexigenic neurons.

Conclusion: GnIH-3 inhibits the reproductive system and concomitantly stimulates food intake in a range of mammalian species.

GnIH is a hypothalamic peptide that is known to inhibit GnRH neurons and gonadotropes, leading to suppression of reproductive capacity in mammals. GnIH has also stimulatory effects on feeding. The relationship between energy balance and reproduction is now well established, but the molecular mechanisms coupling them remain unclear. A high level of GnIH favors food intake over reproduction, while during the follicular phase, a low level of GnIH favors preovulatory LH pulse induced by estrogen and consequent reproductive function. GnIH does not affect sexual behavior, showing that in the brain, mechanisms controlling endocrine and behavioral actions of GnIH are distinct. The pituitary action of GnIH is controversial. However, in the present study, the parenteral administration of GnIH efficiently inhibited reproductive function in ewes without affecting sexual behavior, arguing for a pituitary action without any requirement to cross the blood-brain barrier. The fact that GnIH stimulates food intake without any effect on energy expenditure is of interest and indicates a potential role of this neuropeptide for the treatment of energy-restraint conditions. However, in absence of any genetic mutations in GnIH or physiological investigation, the function of this neuropeptide in humans remains a mystery.

New concerns

Endocrine disruptors in neuroendocrine homeostasis

Endocrine disruptors are of major interest in the field of neuroendocrinology. The two following studies concern two different endocrine disruptors: methylphenidate hydrochloride, a medication that delays puberty in males, and bisphenol A, a chemical compound of food packaging that leads to early puberty and masculinization in females. It is important to determine the mechanisms by which these substances alter pubertal onset in order to better assess their potential risks in humans and also to better understand the mechanisms of pubertal onset.

Pubertal delay in male non-human primates (*Macaca mulatta*) treated with methylphenidate

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Background: Methylphenidate hydrochloride (MPH) is one of the most widely prescribed medications in children. The aim of this study is to evaluate the impact of MPH on pubertal onset in non-human primate.

Methods: Juvenile 2-year-old male rhesus monkeys were treated orally twice a day with vehicle ($n = 10$), 0.15 mg/kg of MPH increased to 2.5 mg/kg (low dose, $n = 10$), 1.5 mg/kg of MPH increased to 12.5 mg/kg for 14 months. Observations were made during 40 months.

Results: Endocrine analyses indicate that serum testosterone level was decreased by MPH and that inhibin B level was increased. MPH reduced testicular volume and delayed testicular descent. However, differences between control and treated groups disappeared in adult monkeys.

Conclusion: Pubertal onset is delayed by MPH in a non-human primate, but this effect is transient and no permanent deficit is seen in adult monkeys.

MPH is widely used as a chronic treatment for attention deficit hyperactivity disorder in children. The effect of this molecule on pubertal onset was interesting to investigate, as it is recognized as a poten-

tial modulator of the pubertal process. This study in juvenile male non-human primates indicates that MPH delayed the testicular development (testicular growth and descent), and decreased plasmatic testosterone level. However, no differences were observed in adult monkeys between control and treated animals. This observation of a transient effect of MPH is reassuring regarding the use of this medication in children. Additional studies must be undertaken to confirm this result in other animal models and in humans and to determine the molecular mechanisms by which MPH delays the pubertal onset in male.

Disrupted organization of RFamide pathways in the hypothalamus is associated with advanced puberty in female rats neonatally exposed to bisphenol A

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Biol Reprod 2012;112:100826

Background: Neonatal exposure to bisphenol A (BPA) alters the timing of puberty onset in female rodents. Disrupted ontogeny of kisspeptin and RFamide-related peptide-3 (RFRP3), which are known to regulate GnRH neurons, could be a consequence of BPA exposure leading to premature puberty.

Methods: Transgenic female Wistar rats expressing enhanced green fluorescent protein (EGFP) in GnRH neurons were exposed to estradiol (E_2), a low dose of BPA (50 $\mu\text{g/kg}$) or a high dose of BPA (50 mg/kg) from postnatal day (PND) 0 through PND 3. Animals were sacrificed on PNDs 17, 21, 24, 28, and 33.

Results: Vaginal opening was advanced by E_2 and a low dose of BPA. On PND 28, E_2 and 50 mg/kg BPA-exposed females had decreased RFRP-3 fiber density and contacts on GnRH neurons. RFRP3 perikarya were also decreased in females exposed to 50 mg/kg BPA.

Conclusion: Premature puberty induced by neonatal BPA exposure seems to result from an accelerated decline of RFRP3 input on GnRH neurons.

Bisphenol A is an endocrine disrupting compound that advances female rodent puberty and induces persistent estrus by unknown cellular and molecular mechanisms. In this study, the authors hypothesize that BPA-induced puberty is linked to abnormal ontogeny of kisspeptins or RFRP3. Treatment with a low dose of BPA during the neonatal period (3 days after birth) induced an earlier vaginal opening associated with a decrease of RFRP3 fiber density, while a high dose of BPA decreased kisspeptin fibers in the arcuate nucleus. The nonmonotonic effect of BPA (a low dose leads to early puberty, while a high dose leads to masculinization) suggests that two distinct mechanisms coexist. E_2 treatment leads to a more advanced puberty than low-dose BPA, without affecting RFRP3 ontogeny. This observation suggests that BPA does not act as a classical estrogenic compound, or that BPA and E_2 act with a different kinetic. This study suggests a new mechanism by which a low dose of BPA modulates ontogeny of RFRP3 leading to advanced puberty. Now, it will be interesting to determine if other endocrine disrupting compounds have comparable effects.

New gene EAP1 and the central regulation of the gonadotropic axis

Hypothalamic EAP1 (enhanced at puberty 1) is required for menstrual cyclicity in non-human primates

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Endocrinology 2012;153:350–361

Background: EAP1 was recently described as a transcriptional regulator playing a role in the hypothalamic control of female reproductive development and estrous cyclicity in rodents. The mechanism of this

function is unknown. EAP1 probably interacts with specific transcription factors. EAP1 was shown to be highly expressed in the medial basal hypothalamus (MBH) of non-human primate. Here, Dissen et al. have studied the hypothalamic function of EAP1 in rhesus monkey.

Methods: Lentiviral-mediated delivery of small interfering (si)RNA directed against EAP1 was used to reduce EAP1 mRNA levels in the mediobasal hypothalamus. The hypothalamic region of the injection was controlled by a MRI-assisted stereotactic procedure. Green fluorescent protein directed by the cytomegalovirus promoter was used to follow transduced cells in the hypothalamus.

Results: Lentivirus-mediated siRNA against EAP1 in the mediobasal hypothalamus resulted in knock-down of EAP1 and in cessation of menstrual cyclicity in female rhesus monkeys undergoing regular menstrual cycles. Neither lentiviruses encoding an unrelated siRNA nor the placement of viral particles carrying EAP1 siRNA outside the mediobasal hypothalamus-arcuate nucleus region affected menstrual cycles.

Conclusion: The region-specific expression of EAP1 in the hypothalamus is required for menstrual cyclicity in higher primates. EAP1 is thus an integral component of a powerful transcriptional-repressive complex which may control reproductive cyclicity by inhibiting downstream repressor genes involved in the neuroendocrine control of reproductive function.

The understanding of the hypothalamic network regulating estrous cyclicity has been recently improved by several original studies. Most of these proteins are receptors, neuropeptides or neurotransmitters. The analysis of EAP1 in the neuroendocrine control of female cyclicity may open new perspectives to understand this very complex process. This study showed that cessation of menstrual cyclicity did not disturb the distribution and the number of GnRH neurons as well as kisspeptin neurons. Thus, other mechanisms must be evoked. The authors proposed that EAP1 silencing probably enhanced activity of neurons involved in the inhibitory control of the estrous cyclicity. Some of EAP1 target genes may be proapoptotic. The silencing of EAP1 may thus result in an increased apoptosis in the hypothalamus. This possible association between apoptosis and gonadotropic deficiency is interesting and should be further characterized.

New mechanism How steroid hormones control behaviors

During early development, sex steroids have a dramatic impact on the establishment of sexually dimorphic brain regions that will further determine typical male or female behaviors. The following article by Xu et al. brings major findings and novel directions on genetic determinisms of sexually dimorphic behaviors whereas the article by Lombardo et al. is the first study to show that in humans, fetal testosterone has an organizing impact on the adult sexually dimorphic brain.

Modular genetic control of sexually dimorphic behaviors

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Cell 2012;148:596–607

Background: It is well known that the development of sexually dimorphic behaviors in vertebrates highly depends on sexual hormones such as estrogen and testosterone. However, the molecular mechanisms responsible for the establishment of the underlying neural circuitry that drives such behaviors still remain to be clearly defined.

Methods and Results: To bring further understanding in these mechanisms, the authors used dual color microarray on adult mouse hypothalamus and amygdala to define sexually dimorphic gene expression patterns between males and females. Further in-situ hybridization analysis of candidate genes revealed sex- and region-specific expressions of hormonally regulated genes. Moreover, among these genes, four were shown to alter distinct sexually dimorphic behavior when individually disrupted (Brs3, Cckar, Irs4 and Sytl4).

Conclusion: These results suggest that sexually dimorphic behaviors such as sexual behavior, male aggression or maternal care are governed by separable genetic programs.

This study is extremely important because it brings further insight in the complex and poorly understood mechanism of how sexual hormones have such a dramatic impact on the organization of the brain. Here, the authors identified 16 dimorphically expressed genes, among which the already known *esr1* encoding estrogen receptor- α , but also novel genes whose functions in sexual behaviors are unknown. Furthermore, the authors used knockout animals to show that specific disruption of some of these genes have very specific impact on sexual behavior. This impact is also different depending on whether the disruption occurs in males or females. For instance, loss of *Cckar* or *Irs4* had no impact on behavior in males but in females, *Irs4* participates in maternal behavior whereas *Cckar* is important for sexual behavior. This study therefore opens novel leads to understand how the environment may alter dimorphic behaviors, for instance through epigenetic modification of such genes. It may also account for subtle behavioral differences between individuals based on polymorphisms or mutations in the target genes. Finally, the microarray used by the authors did not identify several well-described sexual dimorphic genes such as *Kiss1*, androgen receptor or aromatase, showing the limits of such approach but also emphasizing the need of combining different high throughput strategies for the identification of sexual dimorphic genes.

Fetal testosterone influences sexually dimorphic gray matter in the human brain

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J Neurosci 2012;32:674–680

Background: It is well established that during early development of non-human species, testosterone has a major organizing effect on the adult sexually dimorphic brain. However, if such mechanism also occurs in the sexual differentiation of the human brain remains unclear.

Methods and Results: To assess this question, the authors used magnetic resonance imaging in human prepubertal males (aged 8–11) and show that fetal testosterone (FT) levels correlate with the local gray matter volume of specific brain regions such as the right temporoparietal junction/posterior superior temporal sulcus (RTPJ/pSTS), the planum temporal/parietal operculum (PT/PO) and the posterior lateral orbitofrontal cortex (pLOFC). Indeed, FT levels positively correlated with the size of RTPJ/pSTS which was therefore bigger in males than females, whereas PT/PO and pLOFC size negatively correlated with FT and was smaller in males than in females. Other sexually dimorphic regions such as the hypothalamus and the amygdala were unrelated to FT levels although FT positively predicted the size of a nonsexually dimorphic region of the amygdala.

Conclusion: Fetal testosterone appears to act as an organizing mechanism for the sexual differentiation of the human brain.

This is the first study to bring evidence that, in humans, there is a direct correlation between fetal testosterone levels and the gray matter volumes of sexually dimorphic parts of the brain in males. Indeed, by comparing fetal testosterone levels (measured from amniotic fluid samples) to the volume of brain regions in the corresponding prepubertal boys, they observed that FT was likely to organize some specific sexually dimorphic regions of the brain. However, some other well-known sexually dimorphic regions did not seem to be correlated with FT levels, raising the possibility that FT is not the only organizer of the sexually dimorphic brain. Unfortunately, such observational studies are limited, for example one cannot exclude the possibility that FT levels are also correlated to testosterone levels at later stages of development.

FT levels may also not solely account for differences between males and females, but unfortunately, the study is based on only male samples of FT. Then it would be interesting to assess how differences between males and females take place and whether similar correlation between fetal sex steroids levels and local brain regions size is true in females. How FT influences the size of discrete parts of the adult brain remains an open question, especially which are the targeted genes downstream of the FT signaling that are transcriptionnally activated or inhibited.

Novel findings

Autophagy as a major regulator of energy balance

Autophagy is a cellular mechanism that participates in the cellular homeostasis and viability by removing deficient organelles and supplying energy. Recent studies have shown that autophagy contributes to energy homeostasis in the liver, pancreas and adipocytes. However, the role of autophagy in the arcuate nucleus, which integrates all the metabolic signals, remains unknown. Therefore, the three following studies share the same prospect, to show and understand if autophagy participates in the energy balance and metabolism regulation by hypothalamic neurons.

Autophagy in hypothalamic AgRP neurons regulates food intake and energy balance

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Cell Metab 2011;14:173–183

Background: Along with proopiomelanocortin (POMC) neurons, agouti-related peptide (AgRP)-expressing neurons belong to the complex neuronal system that integrates nutritional and metabolic signals in the hypothalamus. Therefore, the authors tested the hypothesis that altered autophagy in AgRP neurons may have a critical impact on the regulation of food intake and energy balance.

Methods and Results: The authors first showed that autophagy was induced in the hypothalamus of starved animals and led to an increase of AgRP expression. Functional studies in the hypothalamic cell line GT1-7 showed that the regulation of AgRP levels is due to an increase of free fatty acids generated by the degradation of endogenous lipids. The authors next generated a mouse line selectively lacking expression of the autophagy gene *Atg7* in AgRP neurons and showed they had impaired feeding and energy balance. Indeed, these mice had reduced body weight and total fat mass without modification of food intake. They further showed that altered autophagy led to a downregulation of AgRP in response to starvation whereas POMC was upregulated.

Conclusion: This study demonstrates that autophagy participates in the neuropeptide response to metabolic status and brings important functional insight into how such mechanisms may occur.

Autophagy was long thought to be induced in almost all tissues, but not in the brain. In this study, Kaushik et al. clearly demonstrate that starvation activates autophagy in the hypothalamus. By using in-vivo and in-vitro models, the authors first show that starvation induced autophagy in the AgRP neurons, mobilizing lipid droplets that stimulate AgRP expression, a neuropeptide that controls appetite. When autophagy was blocked, AgRP mRNA levels failed to rise upon starvation and α -MSH mRNA levels, a cleavage product of POMC, increased. This resulted in decreased animal body weight and total fat mass. Therefore, a high fat diet might trigger a vicious circle of overfeeding, as it might modify lipid metabolism in AgRP neurons. Finally, this study brings novel therapeutic targets for the treatment of obesity.

Loss of autophagy in proopiomelanocortin neurons perturbs axon growth and causes metabolic dysregulation

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Cell Metab 2012;15:247–255

Background: The neurons that produce the proopiomelanocortin (POMC)-derived peptides are part of the hypothalamic melanocortin system, which is a major negative regulator of energy balance. These neurons develop their unique features during neonatal life and begin to form functional neural systems through complex mechanisms such as autophagy, which is a major intracellular mechanism involved in the degradation of proteins and organelles which was recently shown to participate in the energy homeostasis.

Methods and Results: The authors tested the hypothesis that the autophagy-related gene, *Atg7*, a major autophagy gene, is essential to maturation of POMC neurons. Specific deletion of *Atg7* in POMC neurons resulted in higher postweaning body weight, increased adiposity and glucose intolerance. It also caused an age-dependent accumulation of ubiquitin and p62 aggregates in the arcuate nucleus as well as an abnormal development of POMC neuronal projections.

Conclusion: This study brings strong evidence that abnormal autophagy in the hypothalamus can lead to the pathogenesis of obesity and that the expression of *Atg7* in POMC neurons is important for normal metabolic regulation and neurogenesis.

Role of hypothalamic proopiomelanocortin neuron autophagy in the control of appetite and leptin response

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Endocrinology 2012;153:1817–1826

Background: Autophagy has been described as a key cellular mechanism involved in body metabolism in many different tissues. However, its role in POMC neurons, which are key regulators of energy balance, remains unclear.

Methods and Results: This study aimed to examine the role of autophagy in leptin sensitivity, which is critical for the control of body weight. The authors generated a specific mouse line *Atg7*^{ΔPOMC} in which the autophagy-related gene, *Atg7*, is specifically deleted in POMC neurons. Autophagy substrate, p62, was found to accumulate in POMC neurons and colocalized with ubiquitin. *Atg7*^{ΔPOMC} were obese due to increased food intake and decreased exercise, and were unresponsive to leptin. The loss of *Atg7* did not alter POMC neuron number but caused a reduced STAT3 phosphorylation upon leptin challenge.

Conclusion: Overall, these findings show that autophagy in POMC neurons is critical for the control of energy homeostasis and leptin signaling.

These two separate studies used the same experimental strategy to show that autophagy plays a key role in POMC neurons to regulate feeding and energy balance. Targeted deletion of the autophagy gene *Atg7* (encoding ubiquitin E1-like ligase) in POMC neurons led to impaired autophagy in these neurons only and resulted in increased body weight and adiposity as well as perturbations in glucose homeostasis. Altogether, these two studies underline the importance of autophagy in the control of energy balance by POMC neurons processes. POMC neurons are major intermediates in leptin signaling, and both Coupé et al. and Quan et al. show that impaired energy balance is probably due to leptin unresponsiveness in *Atg7*-deleted POMC neurons. Coupé et al. also show that loss of autophagy impairs the ability of POMC neurons to send projections to their target nuclei, showing that autophagy participates in the complex neuronal network that regulates energy homeostasis. *Atg7* is a major autophagy gene, therefore the authors conclude that it is autophagy impairment that leads to energy homeostasis alterations. However, it is unlikely that *Atg7* participates in a single cellular mechanism, as it was shown for other autophagy genes. Moreover, both studies show an increase of p62 accumulation in POMC neurons, and as p62 is involved in many intracellular signal transduction pathways, leptin unresponsiveness might be the result of several impairments of the global intracellular network. The authors focused their study on the arcuate nucleus of the hypothalamus but POMC is also expressed in corticotroph cells of the pituitary and in the hindbrain. Although Quan et al. bring evidence that cortisol levels are not altered in *Atg7*^{ΔPOMC}, one cannot rule out that loss of autophagy in these cells does not impact on the phenotype. Altogether, these two complementary articles bring a major advance in the understanding of the central control of energy balance and underline the importance of autophagy in metabolism regulation.

Uncoupling the mechanisms of obesity and hypertension by targeting hypothalamic IKK- β and NF- κ B

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Nat Med 2011;17:883–887

Background: Recent research on the pathophysiology of obesity has implicated a role for the hypothalamus. However, it remains unknown whether the often-seen coupling of hypertension with obesity can also be explained by hypothalamic dysfunction.

Methods and Results: Overexpression of a constitutively active IKK- β to activate NF- κ B in the mediobasal hypothalamus elevated blood pressure in mice independently of obesity. This form of hypothalamic inflammation-induced hypertension was reversed by sympathetic suppression. Loss-of-function studies further showed that NF- κ B inhibition in the mediobasal hypothalamus counteracted obesity-related hypertension in a manner that was dissociable from changes in body weight. The authors found that proopiomelanocortin (POMC) neurons were crucial for the hypertensive effects of the activation of hypothalamic IKK- β and NF- κ B.

Conclusion: Obesity-associated activation of IKK- β and NF- κ B in the mediobasal hypothalamus in the hypothalamic POMC neurons is a primary pathogenic link between obesity and hypertension. The treatment of hypothalamic inflammation is therefore a new avenue to control and prevent obesity-related hypertension.

Obesity-related hypertension in children is becoming more prevalent around the world as a consequence of widespread childhood obesity. Epidemiologic studies have shown that early hypertension in childhood increases the risk of developing hypertension later in life and an early treatment is important to reduce cardiovascular risk. Endocrine mechanisms, cytokine-related mechanisms as well as neuronal mechanisms have been proposed to explain obesity-related hypertension. In this study, the authors have showed the role of the sympathetic system in the occurrence of obesity-related hypertension in obese mouse fed with a high-fat diet. It is clear that obesity-related hypertension is not only due to one mechanism but their results indicate that treatment oriented toward the NF- κ B system in POMC hypothalamic neurons may represent an original avenue to control obesity-related hypertension.

Evoked axonal oxytocin release in the central amygdala attenuates fear response

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Background: Recently, oxytocin (OT) received increasing attention for its effects on social behaviors. The mechanism of this effect and specifically how it reaches central brain regions is still unclear. In this study, the authors used a very recent and promising in-vivo strategy, the optogenetic model, to analyze this mechanism.

Methods: The authors used recombinant viruses to selectively express channel rhodopsin 2 (ChR2) in OT neurons and then activated ChR2 by blue light to locally induce the release of OT at the axon terminals of OT neurons.

Results: The authors initially validated the optogenetic model using fluorescent markers. Subsequently, they showed projection of OT axons in the central amygdala, a structure involved in OT-mediated fear suppression. Local blue-light induced an endogenous OT release in the central amygdala and decreased freezing responses in fear-conditioned rats.

Conclusion: This study shows the widespread central projections of hypothalamic OT neurons and demonstrates that OT release from local axonal endings can specifically control region-associated behaviors at distance from the hypothalamus.

This study is interesting for two main reasons. The first reason is a strategic one, as it illustrates the optogenetic technique to specifically control neuropeptide release by axonal endings in a specific region of the brain. Optogenetics was declared by *Nature Methods* as the Method of the Year 2010 [Deisseroth K: Optogenetics. *Nat Methods* 2011;8:26–29]. This method is based on the idea that light may be used to experimentally control cell activity by expressing genetically encoded light-sensitive proteins in highly selected cell types. It offers the capacity to control neuronal activity at timescales relevant to the brain's in-vivo physiology. In this study, the authors adapted the method to study OT neuronal effects distal to the hypothalamus. Using virus-directed gene expression, they specifically expressed ChR2 in all hypothalamic OT neurons and at axon endings outside the hypothalamus. As blue-light activation was able to induce OT release, they were able to specifically analyze the effect of OT in the central amygdala. This is the second reason of the interest of this study. The authors showed that endogenous OT has an inhibitory effect in the central amygdala. This effect controls the fear response in fear-conditioned rats. The function of OT in the control of fear response is not new, but this study showed for the first time that this effect is mediated via OT neurons originated from the hypothalamus.

The development of optogenetics is going to completely change our in-vivo approach. It is now possible to specifically control cell activity and therefore should be very helpful to delineate the very complex hypothalamic regulation of the endocrine axis.