# Distribution and relationship of neurons containing feeding-related neuropeptides in the human hypothalamus

PhD Thesis

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## Introduction

The energy homeostasis depends on the balance of the energy intake and energy expenditure. This balance is very tightly regulated by the interaction of the peripheral organs and the central nervous system. The white adipose tissue, pancreas and the gastrointestinal tract send information to the brain about the actual conditions of the energy depots and food consumption through the vagus nerve, but also by secreting hormones into the peripheral circulation.

The discovery of leptin in 1994 drew scientific attention to the importance of humoral communication between the white adipose tissue and the brain in the regulation of the energy homeostasis. Leptin is synthesized and secreted to the circulation by the white adipocytes. The level of leptin in the serum is proportional to the size of energy stores. Thus during fasting, leptin levels are decreased, while obese animals have increased levels of leptin. Exogenous administration of leptin markedly inhibits the food intake and increases the energy expenditure resulting in a loss of body weight. In contrast, the absence of leptin in the ob/ob mice or absence of leptin receptor in the db/db mice results in morbid obesity, type II diabetes, and a reduction in activity, metabolism, and body temperature.

In addition to leptin, a long list of peripheral hormones including insulin, CCK, PYY inhibit food intake and increase the energy expenditure. Currently, however, only one circulating orexigenic hormone is known: ghrelin is synthesized in the wall of the stomach, exert orexigenic effect and its level is increased during fasting. The primary central target of most of these hormones is the hypothalamic arcuate nucleus.

The arcuate nucleus is one of the most important metabolic sensor areas of the brain. Ablation of the arcuate nucleus by monosodium glutamate treatment results in obesity and leptin resistance. The arcuate nucleus contains at least two, distinct populations of neurons that are directly regulated by peripheral feeding related signals, and involved in the regulation of feeding-behavior. The first is a medially located neuronal group that synthesizes and co-contains the two, potent, orexigenic peptides, NPY and AgRP that are inhibited by leptin and stimulated by ghrelin. The second is a more laterally located neuronal group that synthesizes and co-contains the anorectic peptides, α-MSH and CART, that are stimulated by leptin and inhibited by ghrelin.

**Role of the AGRP/NPY neurons in the regulation of energy homeostasis**

The orexigenic neurons of the arcuate nucleus are located in the ventromedial part of the nucleus and express NPY and AGRP. The importance of this neuronal group in the regulation of food intake is clearly demonstrated by the data showing that selective ablation of the NPY/AGRP neurons in adult mice results in profound hypophagia and weight loss.

NPY, a 36-amino-acid amidated peptide and member of the pancreatic polypeptide (PP) family, is one of the most potent orexigenic substances. Central administration of NPY results in a marked increase of food intake and weight gain. In addition, a recent study where NPY expression in the ARC was inhibited by a gene therapy approach showed that the treated animals released 50% less NPY, gained less weight and ate less than the controls up to 50 days after treatment. Since third ventricular administration of NPY potently stimulate food intake, it is believed that the orexigenic effect of NPY is primarily exerted through the hypothalamus. Indeed, focal administration of NPY into several hypothalamic nuclei results in increased food intake, however, NPY is the most potent when it is injected into the PVN or into the neighboring perifornical area.

In addition to its effect on the food intake, NPY also inhibits the energy expenditure while simultaneously inducing lipogenic enzymes in the liver and white adipose tissue. The inhibitory effects of NPY on the energy expenditure are partly mediated through the inhibition of the sympathetic nervous system. NPY decreases sympathetic activity in the brown adipose tissue (BAT) in a dose dependent fashion after injection into the third ventricle or into the paraventricular nucleus (PVN). Since the thermogenesis of BAT is an important component of the energy expenditure, the inhibition of BAT thermogenesis through the sympathetic nervous system is important in the regulation of the energy homeostasis of the animals. NPY also influences the energy expenditure through the regulation of the hypothalamic-pituitary-thyroid (HPT) axis. NPY neurons of the arcuate nucleus directly innervate the hypophysiotropic TRH neurons and decrease the TRH synthesis in these cells. Therefore, increased NPY synthesis and release inhibit the hypothalamic-pituitary-thyroid axis. Since hypothyroidism reduces the basal metabolic rate by 30%, the fall of thyroid hormone levels induced by the

central NPY treatment may be an important component of the effect of NPY on the energy homeostasis.

NPY binds to at least five different $G_i$ protein-coupled receptors of the PP family, including the Y1, Y2, Y4, Y5, and Y6 receptors. However, NPY can exert its central effects only through the Y1, Y2, and Y5 receptors in the rat, as the Y6 receptor is not expressed in the rat, and NPY has negligible affinity for the Y4 receptor, which is primarily a PP receptor. Of these three NPY receptors, only the Y1 and Y5 receptor subtypes are believed to postsynaptic NPY receptors. Activation of both Y1 and Y5 receptors influences the same intracellular pathway, as both receptor subtypes decrease intracellular levels of cAMP *in vitro.*

AGRP, the second orexigenic peptide of the orexigenic arcuate nucleus neurons was discovered based on the an interesting genetic anomaly: in the agouti mice, ectopic overexpression of agouti-peptide in the central nervous system results in obesity despite the fact that agouti-peptide is not expressed in the brain of wildtype mice. Sequence similarity was used to identify, AGRP, a protein that is 25% identical with agouti and expressed in the brain. Within the central nervous system, AGRP is synthesized exclusively in the NPY/AGRP neurons of the arcuate nucleus.

Similarly to NPY, centrally administered AGRP increases food intake and decreases energy expenditure. Central administration of AGRP results in hyperphagia and obesity. In addition, transgenic overexpression of human AGRP in mice also causes obese phenotype. The effect of AGRP on body weight gain is only partly mediated through the increased food intake, because AGRP treated animals that are *pair-fed* to controls, also have increased body weight gain and increased fat accumulation. These data indicate that independently from its effect on food intake, AGRP decreases the energy expenditure. Indeed, both acute and chronic central administration of AGRP decreases the oxygen consumption and the activity of brown adipose tissue even in pairfed animals.

AGRP exerts its effect in the central nervous system as a potent selective endogenous antagonist of melanocortin signaling on the melanocortin 3 and 4 receptors (MC3R and MC4R).

The activity of the orexigenic AGRP/NPY neurons is tightly controlled by peripheral satiety signals. These neurons are known to express the long form of leptin receptor,

insulin receptor and also the receptor of the orexigenic hormone, ghrelin. Fasting results in a marked activation of the synthesis of both AGRP and NPY. Both peripheral and central administration of leptin inhibit the synthesis of these peptides and can completely reverse the fasting induced increase of the AGRP and NPY gene expression. In contrast, administration of ghrelin stimulates these orexigenic neurons.

The AGRP/NPY neurons send axonal projections to the feeding related centers of the brain including the hypothalamic dorsomedial and paraventricular nuclei. Through these projections, the AGRP/NPY neurons can mediate the effects of the changes of peripheral satiety related signals toward other metabolic centers of the brain.

**Role of the POMC/CART neurons in the regulation of energy homeostasis**

The anorexigenic neurons are located in the lateral part of the arcuate nucleus in rodents. These neurons synthesize proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART). The posttranslational processing of POMC results in several biologically active peptides including the melanocortin agonist α-melanocyte-stimulating hormone (α-MSH), β-MSH, γ-MSH and ACTH and the opioid receptor agonist β-endorphin. Among the POMC derived peptides, the role of the α-MSH is the best understood in the field of the regulation of energy homeostasis. However, α-MSH is a ligand of all melanocortin receptors except the MC2R, the primary central actions of α-MSH are exerted through the MC3R and MC4R. Central administration of α-MSH or the MC3/4R agonist melanotan II (MTII) reduce food intake in both wildtype and leptin-deficient *ob/ob* mice. In contrast, the administration of the MC3/4R antagonist SHU9119 alone cases increase of nocturnal and fasting induced food intake, indicating that the melanocortin system exerts a tonic inhibitory effect on the food intake. Genetic ablation of the POMC gene or the MC4R results in an obesity syndrome that is highly similar to the obesity phenotype of the agouti mouse. These obesity syndromes together are called melanocortin obesity syndromes and characterized by hyperphagia, hypometabolism, hyperinsulinemia and increased linear growth. The MC4R knock out mice have been shown to be highly susceptible for increased fat content of diet. Wildtype mice initially increase the caloric intake on moderate fat diet, but then reduce the food intake to normalize the caloric intake. In

contrast, MC4R knockout mice are unable to adjust to the increased fat content of food and remain hyperphagic and hypometabolic even on moderate fat diet that results in profound accumulation of body fat. Mutation of MC4R also results in a similar phenotype in humans and can be found in 4-6% of morbidly obese individuals. Deletion of the MC3R results in a markedly different type of obesity. Despite the fact that the MC3R knockout mice has normal body weight and eat normal amount of food, the fat content of these mice is increased by approximately 40%. In addition, no differences of resting and basal metabolic rate can be measured, but these mice have reduced activity suggesting that reduced energy expenditure may contribute to the increased fat content of these animals.

CART synthesized in the anorexigenic neurons of the arcuate nucleus is also an anorexigenic peptide. Acute administration of CART into the cerebral ventricles induces c-fos in many feeding related areas, decreases food intake, and inhibits the orexigenic effects of NPY, whereas chronic CART administration induces significant weight loss. Conversely, the central administration of CART antiserum increases food intake.

While there is general agreement that icv administration of CART has anorexigenic effects, some discrepancies have been reported. Wang et al. observed inhibition of food intake when CART is injected directly into the PVN, whereas Abbott et al. observed increased food intake after focal injection of CART into either the PVN or arcuate nucleus. Nevertheless, consistent with the role of CART as an anorexic peptide, CART deficient transgenic mice develop obesity when placed on a high caloric diet, and polymorphisms of the 5' flanking region of the CART promoter are associated with obesity .

In the arcuate nucleus, the synthesis of POMC and CART is highly regulated by circulating levels of leptin. Exogenous administration of leptin or elevated leptin levels induced by high fat diet-induced obesity, increases both POMC and CART mRNA in the arcuate nucleus. Conversely, both POMC and CART gene expression is decreased in the arcuate nucleus of leptin resistant fa/fa Zucker rats and leptin deficient ob/ob mice. In the later animal model, POMC and CART mRNA levels can be normalized by leptin replacement.

The actions of leptin is believed to be exerted directly on POMC/CART-producing neurons of the arcuate nucleus, as these neurons express leptin receptors and show evidence of SOCS 3 expression following leptin administration. The POMC/CART-synthesizing arcuate neurons project directly to the PVN and the intermediolateral column of the spinal cord, thereby coordinating the effects of α-MSH and CART to simultaneously regulate food intake and energy metabolism.

**Interaction of NPY/AGRP and POMC/CART neurons**

The two functionally antagonistic neuronal populations of the arcuate nucleus, the AGRP/NPY and POMC/CART neurons, interact with each other at the level of the arcuate nucleus, but also in the terminal field of these neurons.

Morphological studies demonstrated a direct innervation of the POMC/CART neurons of the arcuate nucleus by NPY-IR terminals. However, there is no report about the innervation of the AGRP/NPY neurons by α-MSH-containing axons. The asymmetric nature of the interconnection of the two cell types were also suggested by patch-clamp electrophysiological studies using transgenic mice that express GFP either in the POMC neurons or in the AGRP and NPY neurons. In these studies, while NPY strongly inhibited POMC neurons via the Y1 receptor, the α-MSH analog MTII had no effect on activity of NPY neurons in the arcuate nucleus. Identification of MC3 receptor on AGRP neurons, however, may provide evidence that α-MSH/CART neurons may also influence the AGRP/NPY cells.

Interestingly, the terminal field of the orexigenic and anorexigenic neuronal groups highly overlap innervating the same feeding related neuronal populations. This indicates that the two antagonistic neuronal populations may innervate the very same neurons. Indeed, our laboratory has shown that all hypophysiotropic TRH neurons in the PVN that are innervated by the α-MSH/CART neurons are also innervated by axons of the AGRP/NPY neurons of the arcuate nucleus. In addition, the transmitters of the two neuronal populations interact at receptorial or postreceptorial levels. AGRP acts as an antagonist of α-MSH on the MC3R and MC4R, while NPY can prevent the α-MSH induced CREB phosphorylation.

The strong, multilevel interaction of the two oppositely regulated functionally antagonistic neuronal groups may be critical in the fine tuning of the activity of the feeding related neuronal groups of the brain.

NPY/AGRP and α-MSH-containing neurons also form two distinct neuronal populations in the infundibular nucleus, the homolog of the rodent arcuate nucleus, of the human hypothalamus and densely innervate the PVN of the human hypothalamus. In addition, both neuron populations densely innervate the infundibular nucleus, but it is unknown whether an interconnection of these cells exists in the human brain. In addition, it is unknown whether CART and α-MSH are expressed in the same neuronal population of the infundibular nucleus.

**MCH**

Melanin concentrating hormone (MCH) was first described in fish, where similarly to α-MSH, also regulates the pigmentation, however, MCH and α-MSH have an opposing effect on coloration. In addition to peripheral organs, MCH is also expressed in the hypothalamus in mammals. MCH expressing neurons are located in the lateral hypothalamus and the perifornical area. In mammals, MCH has a potent orexigenic effect when administered centrally. Focal administration of MCH into the arcuate, paraventricular and dorsomedial nuclei markedly increases food intake, while administration to other nuclei (supraoptic nucleus, lateral hypothalamic area, medial preoptic area, anterior hypothalamus and the ventromedial nucleus) did not influence the amount of food consumed by the animals. In hypothalamic slices, MCH activates orexigenic peptides NPY and AgRP, and inhibits the anorexigenic α-MSH and CART expression and release in the ARC. These data suggest that the orexigenic effects of MCH are mediated primarily by neurons of the arcuate, dorsomedial and paraventricular nuclei of the hypothalamus. Leptin negatively regulates the synthesis of MCH in the lateral hypothalamus and perifornical area. In contrast, fasting increases the MCH expression in these neurons. The fasting induced changes of MCH synthesis can be restored by leptin administration.

**Ghrelin and the ghrelin-immunoreactive neuronal system of the hypothalamus**

Ghrelin, a 28 amino acid peptide with an n-octanoyl modification in the Ser3 was described as the endogenous ligand of growth hormone secretagogue receptor (GHS-R). Ghrelin-synthesizing cells were first described in the rat stomach. While the fundus of stomach is the major source of circulating ghrelin, a substantially lower amount is synthesized in many tissues including the bowel, kidney, placenta, blood cells, testicles, ovaries, pancreas, pituitary and the hypothalamus. Ghrelin is more potent GH secretagogue than GHRH, and exerts its effect in a dose-dependent manner. Ghrelin also increases the circulating levels of prolactin, ACTH and cortisol, whereas it has only a negligible impact on the release of other hypophyseal hormones. In addition to its effects on the pituitary, ghrelin was also shown to have a potent orexigenic effect and so far ghrelin is the only known peripheral orexigenic peptide. Circulating levels of ghrelin are elevated during fasting, cachexia or anorexia nervosa, and decreased after feeding. Furthermore, fasting has also been shown to regulate ghrelin synthesis in the hypothalamus. In the arcuate nucleus, administration of ghrelin directly activates the NPY producing neurons, and can antagonize the effects of leptin on these cells. In contrast ghrelin inhibits POMC/CART neurons, but this effect of ghrelin is indirect and mediated through a gabaergic pathway. Therefore, ghrelin is considered as a "meal-iniciating" signal. Peripheral ghrelin may access the brain through vagal afferents, as ghrelin receptors (GHSR-1) are expressed in the sensory neurons of the vagus, but ghrelin also may reach arcuate nucleus directly. GHSR-1 is also widely distributed in the rodent brain. The presence of ghrelin receptor in feeding-related nuclei of the hypothalamus and activation of these regions after ghrelin administration indicate a major role of these brain regions in the mediation of orexigenic effects induced by ghrelin. In addition to be regulated by peripherally synthesized ghrelin, the ghrelin-responsive neurons may also be influenced and regulated by brain-born ghrelin, as it is suggested by the presence of ghrelin mRNA and mature ghrelin in the rodent hypothalamus. Ghrelin-immunoreactive perikarya were described in several parts of the rodent hypothalamus, including a unique location delineating an area among the ventromedial, dorsomedial and paraventricular nuclei. RT-PCR studies show ghrelin expression in the arcuate nucleus. Ghrelin-IR neurons of rodents project to several

hypothalamic nuclei involved in the regulation of energy homeostasis, including the arcuate nucleus, the PVN, the lateral hypothalamus and the hypothalamic dorsomedial nucleus. However, the presence of ghrelin-IR neurons has been demonstrated in the human infundibulum, the projection fields of ghrelin-synthesizing neurons are unknown in the human hypothalamus.

**Specific Aims**

1. To reveal the interconnection between orexigenic NPY- and anorexigenic α-MSH producing cells in the human hypothalamus

2. To determine the presence of CART in the orexigenic and anorexigenic cell populations of the human hypothalamus

3. To map the distribution of ghrelin-IR elements in the human hypothalamus

**Materials and Methods**

*Human tissue preparation*

Diencephalic samples of four adult human individuals with no history of neurological or endocrinological disorders were obtained at autopsy. Tissue samples were taken within 6-24 h after death in accordance with the permission and regulations of the Regional Committee of Science and Research Ethics, Budapest, Hungary (permission number TUKEB 49/1999). The diencephalic blocks were fixed in a mixture of 4% acrolein and 2% paraformaldehyde for 48 h at 4 °C, then cryoprotected in 30% sucrose and frozen on dry ice. Serial 30-μm thick coronal sections were cut parallel to the lamina terminalis with a freezing microtome (Leica Microsystem, Nussloch GmbH, Germany) and stored in a freezing solution (30% ethylene glycol; 25% glycerol; 0.05 M PB) at −20 °C until used.

Three adult male Wistar rats were used to determine whether the conditions used for preparation of human tissues may alter the results of the experiments. The rats were kept under standard environmental conditions (light between 06:00-18:00 h,

temperature 22±1°C, rat chow and water *ad libitum*). All experimental protocols were reviewed and approved by the Animal Research Committees at the Institute of Experimental Medicine of the Hungarian Academy of Sciences.

Rats were euthanized with nembuthal (70 mg/kg BW), then the carcasses were stored at 4°C for 6h. The brains were removed from the skull and processed as described above for human tissues.

Methods used in the thesis are listed in Table 1.

Table 1. Summary of methods

| Experiment | Method |
|---|---|
| 1. Interconnection between NPY/AgRP and α-MSH/CART-IR neuronal groups in the nucleus infundibularis | Double labeling immunofluorescence<br>Analysis with confocal microscope |
| 2. Interconnection between CART-IR neuronal groups and other neuronal populations containing feeding-related peptides in the human hypothalamus | Double labeling immunofluorescence<br>Analysis with confocal microscope |
| 3. Mapping of ghrelin-IR neuronal elements in the human hypothalamus | Immunohistochemistry with DAB labeling |

*Fluorescent immunohistochemistry (experiment 1 and 2)*

Frozen blocks were sliced into sections with a Leica freezing microtome. In order to decrease autofluorescence, Sudan Black staining was applied to sections before anibody was added. FITC- and Cy3-conjugated secondary antibodies (Jackson ImmunoResearch) were used to visualize the immunoreactions. Immunfluorescent samples were analyzed with a Bio-Rad Radiance 2000 confocal microscope.

*Non-fluorescent light microscopic analysis*

In the third experiment, antigens were detected with a peroxidase reaction. Ghrelin-immunoreactivity was visualized with a black colored silver-intensified Nickel-diaminobezidine (Ni-DAB) chromogen.

*Statistics*

Data are presented as standard mean±SEM (standar error of mean). Student T-test was used to compare the size of NPY and α-MSH neurons.

## Results

### 1. Interconnection between α-MSH- and NPY-IR neurons in the hypothalamic infundibular nucleus in humans

NPY- and α-MSH-IR neurons were present in the entire length of the infundibular nucleus of the human hypothalamus. NPY-IR cells were of small to medium size in a range of 14–30 μm (average size: 21.0 ± 0.5 μm) and displayed fusiform or multipolar shapes. The α-MSH-IR cells were significantly larger (26.1 ± 0.6 μm; $P > 0.001$) in a range of 16–36 μm and had multipolar in shape. While the two neuron populations were intermingled in the infundibular nucleus, colocalization of the two peptides was not observed. NPY- and α-MSH-IR axons and their terminals were numerous in the infundibular nucleus. The two axon systems were interwoven in this region and formed reticular networks that hosted the NPY- and α-MSH-IR perikarya and dendrites. The α-MSH-IR neurons were intensely surrounded by NPY-IR varicosities. The vast majority of the α-MSH-IR cells, 97.00 ± 1.00%, was contacted by NPY-containing axon varicosities. In some instances, the NPY-IR varicosities encircled the α-MSH-IR cell bodies. An average of 6.56 ± 1.32 NPY-IR boutons was found on the surface of an α-MSH-IR perikaryon. α-MSH-IR axon varicosities contacted 79.67 ± 8.33% of NPY-IR neurons. However, only an average of 2.27 ± 0.10 α-MSH-IR varicosities was observed in juxtaposition to an NPY-IR neuron.

## 2. Colocalization of CART with NPY, α-MSH, AgRP and MCH peptides in the human hypothalamus

In the infundibular nucleus of the human hypothalamus, numerous α-MSH-IR neurons and relatively less CART-IR perikarya were found in a dense network of axons containing α-MSH or CART-immunoreactivity. No co-localization of these peptides was observed in neurons of the infundibular nucleus. In addition, only single labeled α-MSH and CART axons were observed in both the infundibular nucleus and the PVN. In contrast, in the rat brain processed similar to the human hypothalamic samples, the majority of α-MSH-IR neurons in the arcuate nucleus and α-MSH-IR axons in the arcuate nucleus and PVN also contained CART-immunoreactivity. CART-immunoreactivity was present in a population of NPY-IR perikarya in the human infundibular nucleus. Similarly, CART-immunoreactivity was also observed in neurons containing AGRP, known to be co-expressed with NPY in the human infundibular nucleus. Semiquantitative analyses demonstrated CART-immunoreactivity in 35.7±2.2% of NPY-IR cell-bodies in the infundibular nucleus, whereas CART/NPY neurons formed 54.1±4.6% of CART–IR neurons. CART also co-localized with both NPY and AGRP in numerous axons in the infundibular nucleus and PVN of the human hypothalamus. In the perifornical region of the lateral hypothalamus, colocalization of CART- and MCH-immunoreactivity was observed in both perikarya and axonal processes. While 56.7±3.4% of CART-IR neurons co-contained MCH, CART was present in 44.9±7.1% of MCH neurons in this brain region. Examination of double labeled preparations also revealed that the feeding-related neurons of the infundibular nucleus and the lateral hypothalamus are frequently contacted by CART-IR varicosities. The vast majority of α-MSH, NPY/AGRP and MCH neurons were juxtaposed by CART-IR axons. In most cases, CART-IR varicosities encircled the immunolabeled neurons. All studied parameters were highly similar in the four studied cases independently from the cause of death.

## 3. Distribution of ghrelin-immunoreactive elements in the human hypothalamus

Dense networks of ghrelin-immunoreactive fibers were observed in several areas of the human hypothalamus. Based on the thickness of fibers, two major types of ghrelin-IR axons were seen in the hypothalamus; i.e., thick fibers with large varicosities and very fine fibers with or without small varicosities. A dense network of thick fibers with large varicosities was observed in the caudal part of supraoptic nucleus, in the periventricular and suprachiasmatic nuclei and in the periventricular part of the paraventricular nucleus. A loose network of very fine fibers was detected in the other parts of the paraventricular nucleus, in the ventral perifornical region and in the rostral part of supraoptic nucleus. The periventricular nucleus also contained very fine fibers with small varicosities, in addition to the thick varicose fibers. A network of thick fibers was observed along the medial and lateral zones of the infundibular nucleus, whereas bundles of thick fibers were intermingled with a dense network of thin axons in the central part of the nucleus. The dorsomedial and the ventromedial nuclei were filled with a dense network of very fine fibers. Varicose immunolabeled fibers were also detected in the external layer of the pituitary stalk. While most regions of the mammillary complex lacked ghrelin-IR elements, a few thin fibers were observed in the ventromedial part of the mammillary nucleus. Ghrelin-immunoreactive cell bodies were not detected in the processed human hypothalami.

## Conclusions

We conclude that unlike in rodents, α-MSH and NPY neurons are anatomically interconnected in the infundibular nucleus of the human hypothalamus. However, in contrast to the very dense NPY-IR innervation of α-MSH-IR neurons, NPY neurons are less frequently contacted by α-MSH-IR varicosities. These data suggest an asymmetric, but bidirectional communication between these functionally antagonistic neuron populations.

In contrast to the rodent brain, CART is synthesized in a population of NPY/AGRP neurons and it is absent from the α-MSH-synthesizing neurons in the infundibular nucleus of the human hypothalamus. These data indicate that there are important differences between the hypothalamic feeding regulatory systems of the humans and rodents, and raise the possibility that CART may play a different role in the regulation of feeding in humans than described in rodents.

Our present data demonstrated the dense ghrelin-IR innervation of multiple hypothalamic nuclei including the feeding-related infundibular, paraventricular and dorsomedial nuclei. The findings suggest a definite role of the hypothalamic ghrelin-IR neuronal circuits in central regulation of energy balance in humans.

**List of publications underlying the thesis**

1. Menyhért J., Wittmann G., Hrabovszky E., Keller É., Liposits Z., Fekete C.
   Interconnection between orexigenic neuropeptide Y- and anorexigenic alpha-melanocyte stimulating hormone-synthesizing neuronal systems of the human hypothalamus
   Brain Res, 1076 (2006) 101-105

2. Menyhért J., Wittmann G., Hrabovszky E., Szlávik N., Keller É., Tschöp M., Liposits Z., Fekete C.
   Distribution of ghrelin-immunoreactive neuronal networks in the human hypothalamus
   Brain Res, 1125 (2006) 31-36

3. Menyhért J., Wittmann G., Lechan R.M., Keller É., Liposits Z., Fekete C.
   Cocaine- and amphetamine regulated transcript (CART) is colocalized with the orexigenic NPY and AGRP and absent from the anorexigenic α-MSH neurons in the infundibular nucleus of the human hypothalamus
   Endocrinology. 2007 Sep;148(9):4276-81.

**List of publications related to the subject of the thesis**

1. Zeöld A, Doleschall M, Haffner MC, Capelo LP, Menyhért J, Liposits Z, da Silva WS, Bianco AC, Kacskovics I, Fekete C, Gereben B.
   Characterization of the nuclear factor-kappa B responsiveness of the human dio2 gene.
   Endocrinology. 2006 Sep;147(9):4419-29.

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