THE EFFECTS OF CHOLECYSTOKININ AND ALARIN IN THE REGULATION OF ENERGY HOMEOSTASIS

Ph.D. thesis

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Pecs, 2017

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1. Introduction

Aging is associated with characteristic changes in the regulation of energy balance leading to alterations in body weight and body composition. In the middle-aged, weight gain and obesity presents challenging public health problems, while in the elderly anorexia and the consequent muscle loss (called aging sarcopenia) promotes the development of cachexia and frailty (Petervari et al., 2011a, Kmiec et al., 2013). Increasing incidence of obesity is a woldwide phenomenon. According to data of the World Health Organization (WHO), 39% of adult population was overweight and 13% was obese in 2014, representing a two-fold increase in incidence of obesity since 1980 (WHO, 2016). Obesity leads to serious medical consequences e.g. cardiovascular diseases, diabetes mellitus, infertility. Some types of tumors also show increased incidence. These conditions present enormous public health burden in Western societies, they may accelerate age-related degenerative processes and may lead to lethal consequences (Kopelman, 2000). Aging is one of the most important challenges of our world. According to the WHO, the proportion of the world's population over 60 years will reach 22% by the year 2050 (WHO, 2014). Aging, obesity and sarcopenia appear to take part in a vicious circle, leading to severely impaired quality of life and to increasing world-wide public health care costs (Balasko et al., 2016).

The above mentioned aging-induced tendencies in body weight are shown in other mammals, therefore, in the background intrinsic regulatory mechanisms may also be assumed, that may become pathologic during aging due to unhealthy lifestyle (Scarpace and Tumer, 2001). These regulatory mechanisms play a very important role in the shifts of the balance of neuropeptides influencing food intake and metabolic rate. A number of peptide hormones have been shown to influence the components of energy balance (food intake, metabolic rate, body weight, body temperature, etc.) by having either an overall anabolic (orexigenic and hypometabolic) or catabolic (anorexigenic and hypermetabolic) effect (Szekely et al., 2010). The roles played by these peptides are not consistent in the course of life: in the regulation of energy balance both the role(s) of individual peptides and their interactions change continuously. Several peptide mediators (leptin, alpha-melanocyte stimulating hormone (alpha-MSH)) have been shown to have characteristic age-related alterations. Previous studies demonstrated characteristic age-related shifts in both acute and chronic anorexigenic effects of centrally applied leptin or melanocortins, with strong effects observed in young adult

rats, diminished efficacy in middle-aged rats, and very pronounced effects in old rats, that may contribute to the development of both middle-aged obesity and aging anorexia (Petervari et al., 2011b, Rostas et al., 2016). These changes may be influenced by the nutritional state (Szekely et al., 2012). The altered energy balance in obesity results in accumulation of energy leading to pathological alteration of body weight and body composition especially in middle-aged populations. Another shift in the regulation of energy balance leads to loss of energy in aged population – that affects primarily the mobilisable protein-reserves in the muscles – and it will lead to aging sarcopenia.

The investigation of the effects of regulatory peptide mediators has enormous importance in the discovery of the pathomechanisms of diseases due to altered energy balance (e.g. middle-aged obesity, aging sarcopenia). These neuropeptides play nowadays an important role in therapeutic drug development for obesity (Boughton and Murphy, 2013). This thesis focuses on the role of two neuropeptides, cholecystokinin (CCK) and alarin in the regulation of energy balance. Concerning central (hypothalamic) effects of leptin and alpha-MSH, our team revealed not only characteristic age-related shifts (Petervari et al., 2010), but also their alterations by nutritional states (Soos et al., 2010). In the first part of this study we investigated the question, whether peripheral and central regulatory effects of CCK show similar age-related shifts in rats (as leptin or melanocortins) and how the body composition influences these effects. In the second part of my thesis, the effects of alarin, a newly discovered member of the galanin-peptide family will be analyzed in rats on the regulation of energy balance. Earlier studies reported some orexigenic (food intake increasing) and luteinising hormone stimulating effects of alarin (Boughton et al., 2010, Van Der Kolk et al., 2010, Fraley et al., 2012), but its particular role in thermoregulation and in the regulation of food intake is still unknown according to the literature. The aim of our experiments was to clarify the role of alarin in energy homeostasis. Our results may contribute to the indentification of new drug targets in the treatment or prevention of diseases due to alterations in energy balance.

2. Literature review

2.1 Peptidergic regulation of the energy homeostasis

The regulation of energy balance includes different mechanisms which are responsible for keeping the permanent amount of energy stored in the body in various forms. Energy balance involves regulation of body temperature via heat production (metabolic rate, MR) and heat loss, and that of body weight (BW) via food intake (FI) and MR. This can be described as a dynamic state. The regulatory mechanisms of energy balance form two closely related systems (Figure 1).

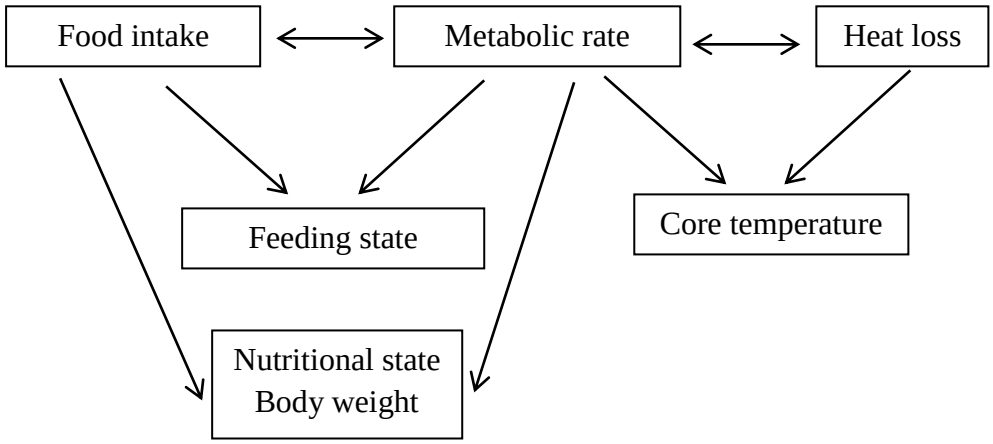

Figure 1. *Main components of energy balance.*

On the short run, the changing level of MR and FI assign the feeding state (hunger or satiety), on the long run, it determinates the nutritional state, the BW. From the point of view of BW, FI and MR might show coordinated changes. Anabolic substances lead to weight gain by increasing FI (orexigenic effect) and by suppression of MR (hypometabolism, usually leading to hypothermia), while catabolic mediators induce weight loss via decrease in FI (anorexigenic) and increase in MR (hypermetabolism usually with hyperthermia) (Szekely and Szelenyi, 2005). In addition, in case of imbalance between MR and heat loss (HL), the core body temperature (Tc) will change (hypothermia, hyperthermia) (Figure 2.).

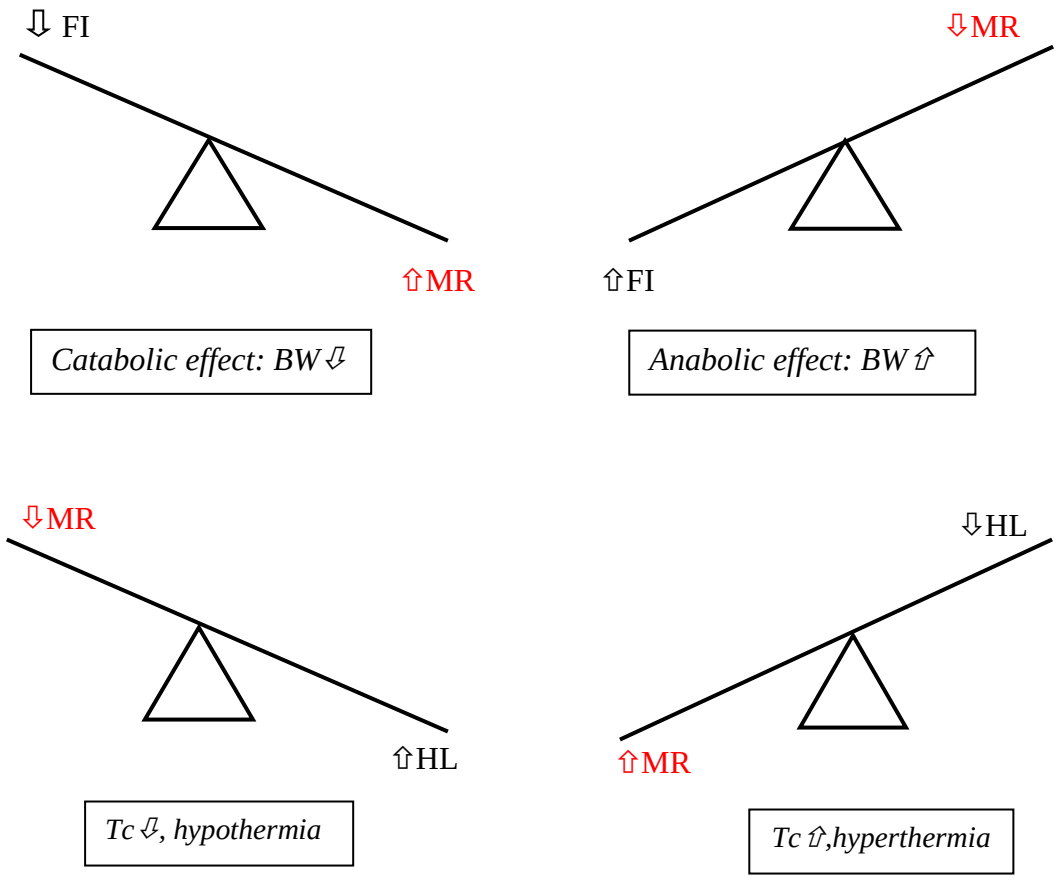

Figure 2. *Coordinated changes of the components of energy balance.*

The members of energy balance affect each other: on the one hand, a change in Tc may influence the FI (e.g. cold-induced hyperphagia), on the other hand, the feeding state may influence the regulation of Tc (e.g. postprandial hyperthermia) (Szekely et al., 2010).

Energy balance appears to be positive (anabolic) until middle-age inducing weight gain, but with further aging, it turns negative (catabolic) resulting in loss of active tissues. Similar trends are seen in humans and other mammals, thus these age-related alterations of energy balance may be of regulatory origin also involving regulatory peptide systems (Scarpace and Tumer, 2001).

Peripherally and centrally produced neuropeptides play an important role in the regulation of energy balance, according to their effects on FI they can be orexigenic or anorexigenic. Orexigenic peptides have often also an anabolic feature, so they decrease MR at the same time to increasing energy reserves in the body. This group of peptides

containes several neuropeptides, e.g. neuropeptide Y (NPY), agouti-related protein, orexins, melanin-concentrating hormone, ghrelin, galanin, endogen opioid peptides. In contrast, some anorexigenic peptides may have also catabolic effects and cause elevation of MR simultaneously, e.g. central action of CCK, alpha-MSH, cocaine- and amphetamine-regulated transcript, corticotropin releasing factor, leptin, insulin, glucagon-like peptide (Szekely and Szelenyi, 2005). Not all regulatory peptides exhibit coordinated effects concerning energy balance. Some peptides affect only certain components of energy balance without coordinated catabolic or anabolic features. Such peptides, e.g bombesin (Tsushima et al., 2003) or neurotensin (Popp et al., 2007) decreases FI as well MR inducing hypothermia.

2.2 Age-related regulatory alterations in energy homeostasis

Instead of a monotonous decline during aging, the effects of the anabolic and catabolic regulators show characteristic alterations, that may result in shifts of their balance. The anorexigenic effect of alpha-MSH after its intracerebroventricular (ICV) injection was strong in young (2-4 months old) rats, then declined in middle-aged (6-12 months old) animals, and it was again increased in the old (18-24 moths old) ones (Petervari et al., 2010). Similar fluctuations were detected concerning the acute thermoregulatory and chronic anorexigenic effects of this hypothalamic peptide (Petervari et al., 2011b, Rostas et al., 2015). In addition, the anorexigenic action of leptin, the main peripheral adiposity signal (that stimulates alpha-MSH-release) also has been shown to have similar age-related pattern upon ICV injection or infusion in rats (Petervari et al., 2014, Rostas et al., 2016). These changes may promote obesity in the middle-aged and aging anorexia in the old population. Nutrition and body composition have been found to influence these age-related changes in effects of alpha-MSH or leptin. Development of leptin-resistance in middle-aged rats was promoted by high-fat diet, while caloric restriction that reduces fat mass may prevent leptin-resistance (Soos et al., 2010, Soos et al., 2011, Petervari et al., 2014).

The question arises, whether effects of other peptides, e.g. CCK, the most important peripheral satiety signal, show similar age-related shifts and whether they may be influenced by body composition. According to the available human data, the fasting plasma levels of CCK are higher in the elderly than in young individuals (MacIntosh et al., 1999, Chapman et al., 2002, Di Francesco et al., 2005, Serra-Prat et al., 2009). In rats, enhanced responsiveness to CCK has been decribed in old age-groups

(Akimoto and Miyasaka, 2010). However, the effect of CCK was not examined yet in middle-aged groups. The first experimental part of this thesis focuses on analysis of age- and body composition-related effects of CCK.

2.3 Cholecystokinin

Cholecystokinin has been discovered by Ivy and Oldberg for over a century as one of the first known hormones, with a function to enhance the motility of the gallbladder (Ivy A.C., 1928a, 1928b). In 1943 Harper and Raper isolated „pankreozymin“ from the small intestine mucosa, which can stimulate pancreas hormone secretion (Harper and Raper, 1943) After a few years it was recognized, that CCK and pankreozymin are the same substance (Jorpes and Mutt, 1966). Its production is stimulated by lipids and proteins (Schwartz and Moran, 1996) in the enteroendocrine I-cells of the proximal small intestine (Raybould, 2007, Dockray, 2009). Cholecystokinin is an anorexigenic peptide (MacIntosh et al., 2001), it inhibits the FI, gastric movement and the production of gastric acid for a while, thus it has an important role in forwarding and digesting the consumed food (Raybould, 2007). This hormone stimulates also the secretion of pancreatic enzymes (Wank, 1995).

2.3.1 Synthesis

Preprocholecystokinin, precursor of CCK includes 115 amino acids (Deschenes et al., 1984). Through posttranslational modification, different forms with various lengths of CCK can be produced (4, 5, 8, 12, 22, 33, 39, 58 amino acids) which are found in several species (Calam et al., 1982, Walsh et al., 1982, Eberlein et al., 1988). The biologically active form is an octapeptide (CCK-8) with o-sulfated tyrosin residue found also in mammals (Ondetti et al., 1970). Cholecystokinin will be inactivated through membrane associated aminopeptidase (Deschodt-Lanckman et al., 1981) and enkephalinase (Zuzel et al., 1985).

2.3.2 CCK receptors

Two types of CCK receptors are known (type 1 and 2). They have very similar amino acid sequences, however they are encoded by different genes (Miyasaka et al., 2002). Both types of receptors are members of the transmembrane G-protein associated superfamily.

Peripheral type-1 CCK receptors (CCK1R-s) are located mainly on the afferent fibers of the abdominal vagus (Smith et al., 1985, South and Ritter, 1988), but they are also found in pancreatic acinar cells, in the gallbladder and in the smooth muscle cells of the pylorus. Although some CCK1R-s have been detected also in the brain: in the nucleus of the solitary tract (NTS), in the area postrema, in the dorsomedial hypothalamus (DMH), in the nucleus interpeduncularis and in the nucleus habenulae (Moran et al., 1986, Hill et al., 1987, Hill et al., 1990a, Hill and Woodruff, 1990b, Hill et al., 1992, Mercer and Lawrence, 1992). The NTS serves as a portal for assessing and integrating visceral afferent signals (including CCK-related signals), while the dorsal motor nucleus provides outbound signals towards neurons of the rostral medullary raphe to influence efferent responses (Blessing, 1997, Berthoud, 2004). This system seems to function on the basis of a within-meal negative feedback satiety signal and it is important mainly in determining the short-term regulation of FI (West et al., 1984, Moran et al., 2006, Balasko et al., 2012) according to the actual feeding state. The role of this system in the long-term regulation of nutritional state (adiposity) is less clear.

The type-2 receptors are located primarily in the central nervous system, in cortex, bulbus olfactorius, nucleus accumbens, hippocampus, amygdala, substantia nigra, dorsale raphe, in the posterior horn of spinal cord and in the hypothalamus, mainly in nucleus paraventricularis (PVN), arcuate nucleus (ARC) and nucleus ventromedialis (VMH) (Gaudreau et al., 1983, Hill et al., 1992, Cheng et al., 1993, Corp et al., 1993). Therefore, in contrast to peripheral administration, centrally applied CCK acts mainly on CCK2R-s of hypothalamic and other nuclei (Mercer et al., 2000). The CCK2R-s are also expressed in vagal afferents. It plays a role also in stress-triggered fear memory and anxiety (Miyasaka et al., 2002). It shows higher homology to gastrin receptors (Wank et al., 1994).

The presence of CCK receptor subtypes shows various patterns in different species of animals, for example in the pancreatic acinar cells of rats CCK1R is predominant, while in pigs CCK2R (Morisset et al., 1996).

2.3.3 CCK and energy balance

By now, it is clear that this gastrointestinal peptide has several functions in forwarding and digesting the consumed food, and also that it causes satiety (Moran et al., 2006). The latter effect indicates that CCK as a peptide hormone of the brain-gut axis can influence cerebral functions related to energy balance. Controversial data have

been reported concerning the contribution of CCK to the maintenance of energy balance. Lo and coworkers demonstrated that CCK knock-out mice proved to be resistant to high-fat diet-induced obesity (Lo et al., 2010). However, this mouse strain also showed impaired fat absorption and enhanced metabolic rate that some authors attribute to their genetic background (Lacourse et al., 1999). Lack of CCK may have also contributed to the fat malabsorption due to impaired gallbladder function. Although such studies do not contradict the potential importance of CCK in the regulation of energy balance, they demonstrate the complexity of CCK effects.

2.3.3.1 Food intake and satiety

Food intake enhances CCK production not only in the upper gastrointestinal tract but also in the hypothalamus (Schick et al., 1990, Schick et al., 1994), with obvious central effects in this latter case. Although peripheral CCK has certain central actions, the central functions of CCK of peripheral or central origin may not be identical. The peripheral administration of CCK induces satiety through CCK1R (Della-Fera and Baile, 1979, Shiraishi, 1990) in rats and also in other animals (Moran and Kinzig, 2004), thus the „meal size“ will be smaller. The CCK1R knockout mice eat larger portions from the chow, but they leave longer timeintervals between meals, so the cumulative FI will be the same (Donovan et al., 2007). In rats with CCK1R deficiency [OLETF (Otsuka Long Evans Tokushima) rats] hyperphagia and obesity develop (Bi et al., 2007). Activation of central receptors (both CCK1R and CCK2R) may also decrease FI (South and Ritter, 1988, Shiraishi, 1990, Hirosue et al., 1993, Akimoto and Miyasaka, 2010).

Regarding activation of the afferent vagus, CCK interacts with other peptides, for example it has antagonistic effect on orexigenic ghrelin (de Lartigue et al., 2007), but it has a positive interaction with leptin (Peters et al., 2006). Furthermore, circulating CCK facilitates the transport of leptin through the brain-blood barrier (Cano et al., 2008).

2.3.3.2 Thermoregulation

Peripherally applied CCK in pharmacological doses elicited hypothermia (Kapas et al., 1987) presumably by a vagal reflex causing hypometabolism, skin vasodilation and consequently increased heat loss, independent of the role of CCK in the regulation of FI and energy balance. This hypothermia can be abolished with CCK1R antagonist MK-329, while CCK2R antagonist L-365,260 had no effect (Rezayat et al., 1999), thus the effect seems to be mediated through CCK1R.

Centrally injected CCK is known to induce fever-like coordinated changes in energy balance: an increase in MR, a decrease in HL, an elevation of Tc (Szelenyi et al., 1994), and it also evokes anorexia (Gibbs et al., 1973). Yoshimatsu and his coworkers described an increased sympathetic activity of brown adispose tissue after central CCK injection (Yoshimatsu et al., 1992). In 1973, lipopolisaccharide (LPS)-induced fever was described, which was accompanied by enhanced interscapular thermogenesis in brown adipose tissue (Szekely et al., 1973). Later it was shown that in endotoxin fever CCK2R-s are also involved (Szekely et al., 1994).

Cholecystokinin-induced hyperthermia can be abolished by CCK2R antagonist L-365,260, but it could not be decreased by CCK1R antagonist L-364,718 (devasepide) (Szekely et al., 1994) nor by pretreatment with the cyclooxygenase(COX)-inhibitor indomethacin (Szekely et al., 1994). The fever induced by LPS or interleukin-1 could not be prevented by CCK1R antagonist (Martin et al., 2000). In contrast, fever elicited by prostaglandin E1 (PGE_1), could not be influenced by pretreatment with CCK2R antagonist in rats (Szelenyi et al., 1994).

2.4 Members of the galanin peptide family

2.4.1 Galanin

The peptide family got its name from galanin isolated first from pig small intestine extract in 1983 (Tatemoto et al., 1983). Galanin contains 29 amino acids in rats (30 in humans), it was named from the glicin at the N-terminal and from the alanin amino acid at the C-terminal. Galanin is synthesized primarily as preprogalanin which is encoded by GAL gene (Evans et al., 1993, Hokfelt and Tatemoto, 2010). From a biological aspect, the first 15 amino acids have the greatest importance proven by the conservation of this sequence in most species (Webling et al., 2012).

For its effects the G-protein coupled galanin receptors (Gal-R) are responsible which have 3 types (Gal-$R_{1\text{-}3}$) (Mitsukawa et al., 2008). By examining the tissue expression of the GAL gene, it was established, that galanin is mainly found in the gastrointestinal tract and in the central nervous system (Kaplan et al., 1988). The Gal-R-s are also found in the nuclei of hypothalamus that regulate energy homeostasis, e.g. PVN (Gal-R_1, Gal-R_2, Gal-R_3), area postrema, ARC (Gal-R_2) and VMH (Gal-R_3) (Mitchell et al., 1997, Mitchell et al., 1999, Gundlach et al., 2001, Mennicken et al., 2002).

The role of galanin in the regulation of energy balance as an orexigenic mediator has been shown in earlier studies after its disovery and, later on, it was confirmed by further experiments (Kyrkouli et al., 1986, Leibowitz, 1998, Crawley, 1999). The peptide has been shown to induce FI and also to enhance alcohol intake (Lewis et al., 2004) especially in animals on high-fat diet. Since high-fat diet and alcohol consumption stimulate the release of galanin, the existence of a non-homeostatic, positive feedback circuit was suggested between galanin, high-fat diet and alcohol consumption, which may play a role in the development of obesity (Leibowitz, 2005).

2.4.2 Galanin message-associated peptide and galanin-like peptide

The second member of the family is the 60-amino-acid containing galanin-message associated peptide , which is also encoded by the GAL gene (Hokfelt and Tatemoto, 2010). Only few references can be found in the literature about the function of this peptide. Its distribution in tissues is similar to galanin (Hokfelt et al., 1992). Its role in pain sensation was proven early (Xu et al., 1995), its antifungicid features were described more recently (Rauch et al., 2007, Holub et al., 2011).

Galanin-like peptide (GALP) containing 116 amino acids was isolated from pig hypothalamus in 1999. It could induce a similar reaction as galanin in a membrane preparation of cells containing Gal-R_2, therefore, it is called galanin-like peptide (Ohtaki et al., 1999). Its amino acid sequence is partly similar to that of galanin, but it is the product of preproGALP (Hokfelt and Tatemoto, 2010). Although the sequences of the two peptides show some homology, they are encoded by different genes on different chromosomes. In contrast to galanin, the expression of GALP can be proven only in the ARC (Larm and Gundlach, 2000). Hovewer, GALP-positive neurons have abundant connections with surrounding structures. Both orexigenic and anorexigenic signals take part in the regulation of GALP-positive neurons (Shiba et al., 2010).

The regulatory role of GALP has been recently suggested in energy homeostasis. GALP is a neuropeptide that has complex effects on energy balance and shows species-specificity. In mice, it decreases FI upon acute and chronic administration (Krasnow et al., 2003, Kauffman et al., 2005). In contrast, in rats GALP induces orexigenic actions (Palkovits, 2003). The FI-related effects of GALP have two phases in rats (Man and Lawrence, 2008a). Following the orexigenic effect in the first hour, anorexigenic effects appear, resulting in decrease of 24-h cumulative FI and in loss of BW (Lawrence et al., 2002). In the background of this early orexigenic effect, release of NPY from the DMH was suggested (Kuramochi et al., 2006). Although NPY presents a similar FI and BW reduction after 24 hours, as shown by our previous work, a NPY infusion causes manifest obesity (Beck et al., 1992, Szekely et al., 2005). Thermoregulatory actions of GALP and NPY seem to be contradictory. While NPY decreases Tc acutely (Szekely et al., 2005) GALP induces Tc elevation through prostaglandin-mediated pathways (Lawrence et al., 2002, Kageyama et al., 2013).

Although the affinity of GALP is the highest to the Gal-R_3 (Lang et al., 2005), its effects concerning the regulation of energy balance are independent of this receptor. Some data suggest the existence of a still undiscovered receptor (Man and Lawrence, 2008a). The most recent studies demonstrated anti-obesity effects of GALP in obese mice after intranasal administration of the peptide (Kageyama et al., 2016).

2.4.3 Alarin

Alarin, named after the C-terminal residue alanine and its N-terminal residue serine, is a 25 amino-acid peptide. It is the newest member of the galanin peptide family, found first in gangliocytes of human neuroblastic tumors (Santic et al., 2006). It is an alternative splice variant of the GALP mRNS. It shares only 5 conserved amino acids at the N-terminal region with GALP, the remaining 20 amino acid sequence shows no similarity. Alarin does not contain the 13-amino acid sequence that is found in the other members of galanin peptide family (Hokfelt and Tatemoto, 2010). It is one of the possible reasons why alarin shows no affinity for the Gal-R-s, but alarin receptors have not been discovered yet (Santic et al., 2007, Boughton et al., 2010). Alarin has also been shown to be localized around the blood vessels with vasoactive actions (Santic et al., 2007) and may have a role in ocular blood flow regulation (Schrodl et al., 2013). In addition, it increases the secretion of luteinizing hormone (LH) in male mice (Fraley et al., 2012), has antidepressant-like (Wang et al., 2014, Wang et al., 2015, Zhuang et al., 2016) and antimicrobial (Wada et al., 2013) effects. The potential role of alarin in the regulation of energy balance is raised by its immunoreactivity in the appropriate murine brain regions controlling FI, metabolism and thermoregulation: such as the ARC, DMH, lateral hypothalamus and PVN of the hypothalamus and the preoptic area (Eberhard et al., 2012). In the rat brain alarin was expressed by cells in the posterior hypothalamus (Eberhard et al., 2007) and in locus coeruleus that receives projections from the hypothalamus (Van Der Kolk et al., 2010). Intracerebroventricular injection of alarin significantly increased the expression of the immediate early gene *c-fos*, a marker for neuronal activation in different brain regions including the PVN, DMH and the ARC of male rats (Van Der Kolk et al., 2010). In accord with these observations, some earlier *in vivo* studies raised also the possibility that alarin may participate in the regulation of FI: they described orexigenic effects of alarin (Boughton et al., 2010, Van Der Kolk et al., 2010, Fraley et al., 2012, Fraley et al., 2013). However, this orexigenic effect appears to be relatively weak compared to that of a major hypothalamic orexigenic mediator, NPY (Boughton et al., 2010). Effects of alarin on spontaneous nighttime FI or on fasting-induced re-feeding have not been fully investigated (Boughton et al., 2010, Fraley et al., 2013).

The first 5 amino acids may be responsible for the FI-related and LH-secretion inducing effects of the peptide, because after enzymatic splicing of this sequence, the truncated alarin is able to antagonize these effects (Fraley et al., 2013).

Regarding another feature of energy balance, thermoregulatory effects of alarin were also investigated: previous reports failed to reveal any change in body temperature in freely moving mice (Fraley et al., 2012, Fraley et al., 2013) or any change in oxygen consumption (indicating MR) in freely moving rats upon a central alarin injection (Van Der Kolk et al., 2010).

3. Aims

3.1 CCK in the regulation of energy balance

In the course of aging energy balance changes in two phases: first age-related obesity (Scarpace et al., 2000) appears followed by aging anorexia and weight loss (Chapman et al., 2002).

Based on the earlier observed age-related shifts in the responsiveness of central catabolic melanocortin system (Petervari et al., 2010, Petervari et al., 2011b) and in that of its peripheral regulator, leptin (Petervari et al., 2014, Rostas et al., 2016), we can hypothesize that the catabolic effects of CCK does not change in a linear fashion during aging. A diminished effect could promote middle-aged obesity, while later on, an enhanced action of CCK might contribute to aging anorexia. This late appearing rise in CCK action is also suggested by the literature (Ohta et al., 1995, MacIntosh et al., 2001, Di Francesco et al., 2005, Serra-Prat et al., 2009), but to date, the effect of this peptide has not been investigated in middle-aged groups, that could demonstrate a similar age-related fluctuation in the activity of this catabolic peptide as seen in case of other catabolic systems. Nutritional state has been shown to modify the age-related changes in the effects of the earlier investigated peptides (Scarpace et al., 2000, Scarpace and Tumer, 2001, Gabriely et al., 2002, Petervari et al., 2014), therefore, we hypothetized that caloric restriction or high-fat diet could also influence the effects of CCK.

The aim of the present study was:

1) to investigate, how central CCK-related effects may contribute to changes of energy balance in the course of aging.
2) to examine, how peripheral CCK-related effects may contribute to changes of energy balance in the course of aging.
3) to test, whether nutritional state can influence the effects of CCK.

3.2 Alarin in the regulation of energy balance

Although an acute orexigenic action of alarin has been described in rats and mice (Boughton et al., 2010, Van Der Kolk et al., 2010, Fraley et al., 2012) its complex contribution to the regulation of energy homeostasis also involving thermoregulation is still unknown.

Previous reports failed to reveal any change in body temperature in freely moving mice (Fraley et al., 2012, Fraley et al., 2013) or any change in oxygen consumption in freely moving rats upon a central alarin injection (Van Der Kolk et al., 2010). Because of its orexigenic actions and due to the presence of the peptide in main hypothalamic regulatory nuclei, we assumed first an anabolic character, hypometabolic/hypothermic thermoregulatory actions of this peptide. However, our pilot studies (as reported in our conference abstract, see (Petervari et al., 2013)) raised the possibility of its catabolic rather than anabolic character. Our present study focused on the detailed analysis of the complex effects of alarin on energy homeostasis in Wistar rats with special emphasis on FI and thermoregulation.

1) First we planned to clarify the thermoregulatory actions of alarin injected ICV into the right lateral ventricles of rats.
2) We also aimed to test the effects of the truncated alarin: we hypothetized its antagonistic effect also concerning thermoregulatory functions.
3) We aimed to investigate the mechanisms of the thermoregulatory actions of alarin. In case of a coordinated hypermetabolic/hyperthermic response we assumed a prostaglandin-mediated reaction.
4) If alarin induces hypermetabolic/hyperthermic reaction, then we could hypothesize, that despite the earlier reports, alarin may act as a catabolic mediator and would suppress spontaneous or re-feeding FI.

4. Methods

4.1 Animals

Male rats from the colony of the Institute for Translational Medicine were used in the experiments. After weaning the animals were kept individually in plastic cages (375x215 mm, height 149 mm, covered by steel grid and equipped with feeder and bottle container) with some wood-chip bedding at an ambient temperature of 22-25 °C.

The lights were on between 06.00-18.00 h. Standard rat chow (CRLT/N rodent chow, Szindbád Kft., Gödöllő, Hungary, 11 kJ/g) and tap water were continuously available (food but not water was removed for a 24- or 48-h period in some groups). For the experiments on the effects of CCK, normally fed (NF) Wistar rats different age-groups have been established.The applied animal groups and corresponding human age groups are represented in Table 1. (The maximal life-span of our colony reaches 30 months, about 50% of rats survive 26 months, but after the age of 24 months surgical interventions are difficult.)

Animals age	Group	Human age
2 months	NF2	juvenile
4 months	NF4	young adult
6 months	NF6	younger middle-aged
12 months	NF12	older middle-aged
18 months	NF18	aging
24 months	NF24	old

Table 1. *Different age-groups of normally fed (NF) animals represent corresponding human age-groups.*

Some animals were caloric-restricted (CR) from age 2 months onwards: they received 2/3rd of the normal daily amount of standard chow (16 g/day), with vitamin and mineral supplementation and unlimited water intake. Some other 6 and 12-months-old rats were made obese by using a high-fat diet (HF, using Diet Induced Obesity Rodent Purified Diet with 60% Energy from Fat, IPS TestDiet®, 21.6 kJ/g) from age 2 months.

For the experiments on the effects of alarin, young age-groups of male Wistar and Long-Evans rats also from the Colony of the Institute for Translational Medicine were used. For the investigation of regulatory peptides affecting FI in our laboratory 3 months old adult male Wistar rats are usually used. These young adult animals have already finished the period of rapid growth. However, earlier studies, investigating the FI-related effects of alarin, tested younger juvenile (6 weeks old) Long-Evans rats (Van Der Kolk et al., 2010). Such animals have not finished the period of rapid growth and

they may show altered FI-associated responses to regulatory peptides (Szekely et al., 2012). Therefore, we tested FI-related effects of alarin in our automated FeedScale system using 3 months old young adult male Wistar rats as well as juvenile, 6 weeks old Wistar and Long-Evans groups.

For 10-14 days before and also during the experimental procedures rats that participated in the assessment of food intake received a powdered form of their respective types of chow and were transferred to an automated FeedScale system which allowed continuous recording of their food consumption and prevented food hoarding. The powdered version of the high-fat diet contained 10% normal powdered chow admixed to the powdered high-fat pellets (20.54 kJ/g). Body weight and spontaneous daily food intake were measured every day at 09.00 h – thereby the animals were also accustomed to regular handling. Rats used in the analysis of metabolic rate and body temperature were habituated for at least a week prior to experiments to semi-restraining boxes in which they were able to move somewhat forwards and backwards but not to change the head-to-tail position.

All experimental procedures and interventions were undertaken according to the general rules of the University of Pecs Ethical Committee for the Protection of Animals in Research. In general, the rules of this Committee are in accord with the main directives of the National Ethical Council for Animal Research and those of the European Communities Council (86/609/EEC). Special permission: BA 02/2000-11/2011, valid for 5 years.

4.2 Surgeries

After about 1 week adaptation period to the experimental settings, surgical interventions were performed under intraperitoneal (IP) ketamin + xylazine [78 mg/kg (Calypsol, Richter) + 13 mg/kg (Sedaxylan, Eurovet)] anesthesia. Animals received also 2 mg intramuscular gentamicin for the prevention of infections. A 22-gauge metal cannula was stereotaxically implanted into the right lateral cerebral ventricle (parameters: 1 mm posterior and 1.5 mm right lateral to bregma, 3.5 mm ventral to dura; coordinates according to the Rat Brain Atlas, (Paxinos and Watson, 2006). The cannula was fixed to the skull by dental cement with the help of 2 miniature screws into the bone (Figure 3). The lumen of this cannula was regularly closed by a stylet – at injections the stylet was replaced by a fitting 28-gauge injection cannula that was connected to a pp10 polythene tube for remote ICV injections. For the *in vivo* testing of

its appropriate location, a few days after the implantation angiotensin II (Sigma, A9525, 20 ng/5 μl) was injected ICV through a pp10 polyethylene tube attachment and the subsequent water consumption was measured. The test was accepted to be positive, if at least 5 ml water was consumed by the animal within 30 min, and the location of the cannula was presumed to be right.

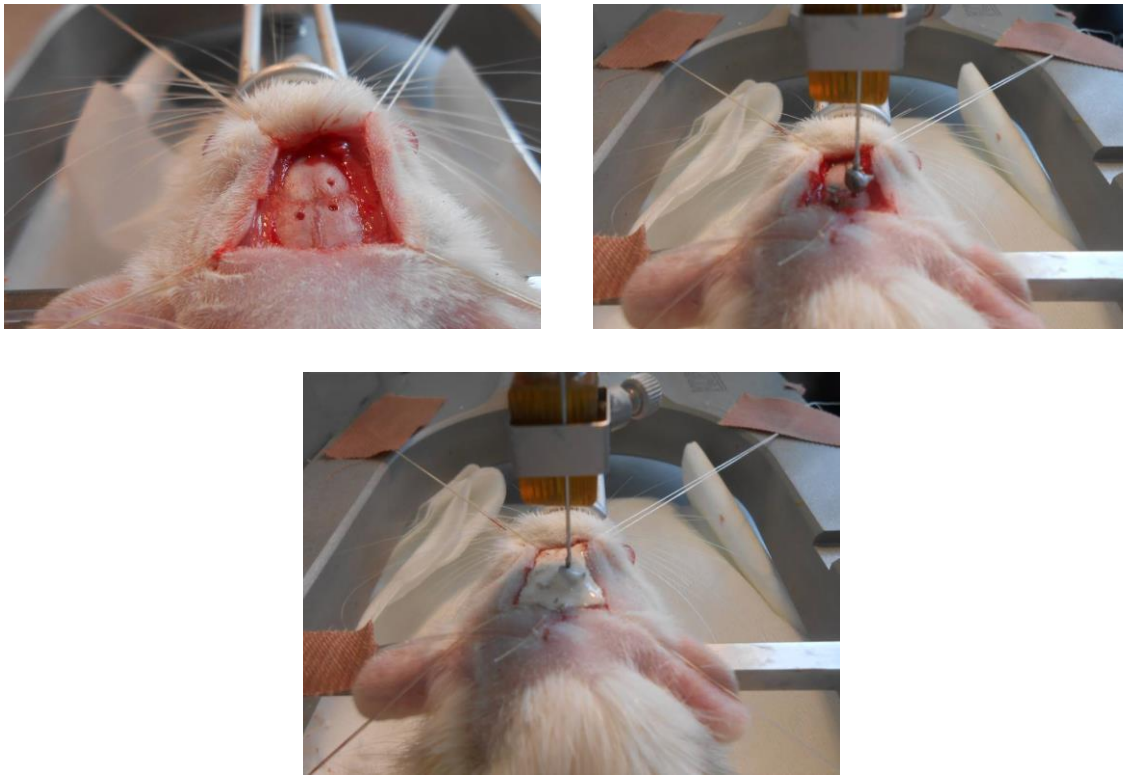

Figure 3. *Steps of the implantation of the ICV cannula into the right lateral ventricle of the brain.*

For IP injections, before the tests, a polythene tube was acutely inserted into the abdominal cavity through a needle under ether anesthesia; after withdrawal of the needle, the tube tunneled under the skin was exteriorized and fixed at the nape.

Following experiments, the animals were euthanized by IP urethane (3-5 g/kg, Reanal). Brains were removed and fixed, the injection sites were checked macroscopically by coronal sections. Rats with inappropriately located cannula were excluded from the analysis. Simultaneously, the left retroperitoneal and epididymal fat pads were removed and weighed, along with the tibialis anterior muscle, as indicators of body composition (Soos et al., 2010). All body composition indicators were calculated for 100 g body weight.

4.3 Substances applied

Cholecystokinin-8 (Bachem) or solvent pyrogen-free saline (PFS) was administered either by direct IP injections at a dose of 5 μg (4.4 nmol) in a volume of 0.5 ml for assessment of anorexigenic effects (Smith and Gibbs, 1998), in other cases at a high dose of 100 μg (88 nmol, as applied in earlier studies, (Kapas et al., 1987) in a volume of 0.1 ml in metabolic studies, or by ICV injections at a dose of 500 ng (0.44 nmol) (Blevins et al., 2000) in a volume of 5 μl for both anorexigenic and metabolic tests. In the analysis of anorexia the injections were given 5 min prior to presentation of food, while in the metabolic studies CCK was injected after the animals reached a thermal steady state (usually 60-90 min after closing the metabolic chamber). For measurements of metabolic rate and body temperature, the ways of CCK administration were slightly different.

Synthetic full-length alarin (alarin 1-25, MW: 2820.19), its truncated (alarin 6-25Cys, MW: 2389.74) and a scrambled forms were custom synthesized by GL Biochem (Shanghai, China). All peptides were dissolved in PFS and given in 5 μl volume ICV. To test dose dependence, alarin was administered at doses of 0.3, 1, 3 or 15 μg (0.1, 0.3, 1 or 5 nmol) alarin at 9.00 h (i.e. early in the inactive phase of the circadian activity) at various environmental temperatures. The highest dose of alarin (90 nmol) caused several of the animals to appear unwell immediately following the injection (Boughton et al., 2010). In these experiments all doses were given to the same group of animals, but the order of doses and that of ambient temperatures were randomized. At least five days elapsed between such tests. In other tests alarin 6-25Cys (2.5 μg) was administered with or without full-length alarin (3 μg) to investigate its potential antagonistic thermoregulatory effects. We also tested the effects of a scrambled alarin (3 μg) containing the same amino acids as alarin, in random order. In the experiments different doses of the peptides filled a 5 μl volume of the proximal end of the 20-25 cm-long pp10 polyethylene tube that was attached to the injection cannula, while the rest of the tube was filled with PFS. A small bubble separated the substance from the PFS in the distal part of the tube. Injecting 5 μl saline at the distal end of the tube resulted in remote ICV delivery of the substance without causing any discomfort to the animal. To test the potential peripheral actions of the applied doses of full-length alarin, they were also administered IP.

In order to investigate the potential prostaglandin-mediated mechanism of the hyperthermic effect of alarin, indomethacin (Sigma, I7378), a non-selective COX inhibitor and meloxicam, a relatively selective COX-2 inhibitor were applied through an IP inserted polyethylene tube 30 min prior to ICV alarin injection. Indomethacin was dissolved in Tris-HCl (0.2 M, pH 8.2), diluted in PFS and injected at a dose of 2 mg/kg (Werner et al., 2006). Meloxicam (Sigma, M3935) was dissolved in 10% ethanol and administered at two different doses: 1 or 2 mg/kg. The prostaglandin-synthesis attenuating effect of subcutanously (5 mg/kg) or intramuscularly (0.25 mg/kg) injected meloxicam has been proven in earlier animal experiments (Mohn et al., 2001, Knorr et al., 2010).

In thermoregulatory tests, the successful ICV administration of alarin was checked by an injection of prostaglandin E_1 (PGE_1, Sigma, P-5515, 100 ng in 5 μl volume, filled in the same pp10 polyethylene tube and separated by a small bubble from alarin) 180 min after the ICV injection of alarin. The test was positive if a prompt hyperthermia developed.

In FI-related experiments, NPY (Bachem, H6375) was given ICV at 5 μg dose in 5 μl, as a positive control.

4.4 Measurement of the thermoregulatory effects and the metabolic rate

Measurements took place between 09.00 h and 15.00 h, and during this period the animals could not eat or drink. For administration the materials an injection cannula inserted into the chronically preimplanted ICV cannula was connected to a 20-25-cm-long pp10 polythene (Portex) tube (Szelenyi et al., 1994). The tube contained at the cranial end the peptide in a volume of 5 μl separated by a small bubble from the PFS filling the rest of the tube, which was closed and exteriorized together with the thermocouples. At injections, 5 μl PFS was slowly injected at the outer end of the tube, thereby the substances were injected ICV without disturbing the animal.

For IP injections the cannula was acutely inserted (through the lumen of a needle) prior to the experiment to the abdominal cavity, fixed by sticky tape and the animal was placed into the restraining box. Both the cannula and the thermocouples were exteriorized similarly as described previously.

During experiments the rats were semi-restrained in a cylindrical wire-mesh confiner in which they could not turn around but their other movements were not

prevented. Having been accustomed to the confiner, semi-restriction did not cause particular stress to the animals (Figure 4).

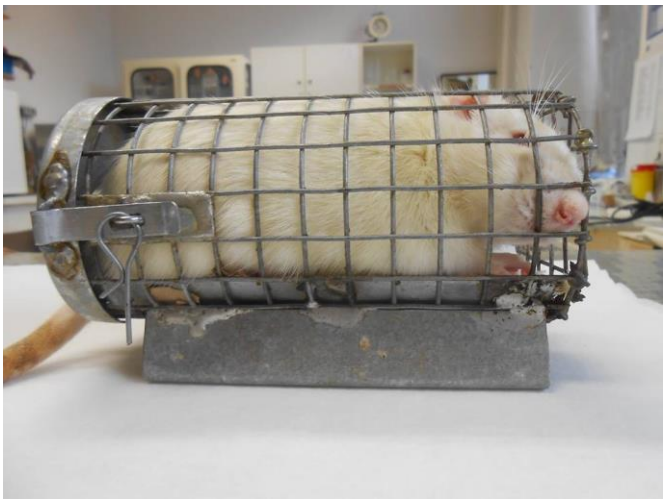

Figure 4. *Wistar rat during accustoming to the cylindrical wire-mesh confiner*

Together with this cylinder, they were placed into an open-circuit metabolic chamber (Figure 5), which in turn, was immersed into a thermostatically controlled water-bath to secure a standard (from thermoneutral to cold) ambient temperature (Ta) in the chamber. At a Ta of 28 °C regularly vasodilation is observed, at 25 °C there is vasoconstriction and no fluctuations in tail skin temperature (concept of thermoneutrality by (Romanovsky et al., 2002), but – in contrast to the cold 20 or 15 °C – they allow to evoke either skin vasodilation or heat loss. On the other hand, at the colder Ta values the cold-induced hypermetabolism allows the observation of a possible hypothermic effect. Four metabolic chambers were used simultaneously. Copper-constantan thermocouples were attached to the rats for measuring colonic (core) and tail skin temperatures (Tc and Ts, respectively): these – together with the thermocouple for the chamber – were exteriorized from the sealed chamber. All temperature data were collected by a Digi-Sense 12-channel scanning Benchtop thermometer (Cole-Parmer) for electronic evaluation.

Figure 5. *Oxymax indirect calorimeter system with metabolic chambers complemented with thermocouples and Benchtop thermometer for measuring thermoregulatory and metabolic functions.*

In metabolic chambers of an Oxymax indirect calorimeter (Columbus, OH) oxygen consumption (VO_2), respiratory quotient (RQ) and carbon-dioxide production (to determine MR) were measured by the help of an Oxymax gas analyzer (Columbus, OH) and the data were electronically processed.Total heat production was judged from the metabolic rate measured by indirect calorimetry, on basis of gas analysis of the air samples leaving the ventilated chamber and expressed as VO_2 in ml O_2/kg/min.

Heat loss state ("heat loss index", HLI, as used in earlier studies; (Romanovsky et al., 2000) was assessed from the relationship of the three monitored temperatures [HLI = (Ts-Ta) / (Tc-Ta)]: Ts values approaching Ta (HLI near 0) suggested vasoconstriction as a sign of heat conservation state, while those Ts values nearer to Tc (HLI near 1) suggested vasodilation as an early manifestation of general enhancement of heat loss activity.

4.5 Measurement of the food intake

For two weeks before the experiments rats were transferred into the automated Feed-Scale system (Columbus, OH) to get habituated to the environment and to the powdered form of rat chow (Figure 6). This powdered form of chow prevented food hoarding. The animals were kept in individual cages of the system. A special digital scale under the cage provided precise automated measurement and continuous recording

of the amount of consumed food. Data were registered every 10 minutes, in case of the measurement of the cumulative 24-h FI, data were collected every 30 minutes.

Figure 6. *The FeedScale-system allowing precise measurement of food intake.*

The anorexigenic responsiveness to IP or ICV CCK injections was assessed in a number of rats (6-8 rats per group) from different populations according to age and nutritional state via measuring their inhibitory effects on 3-h cumulative FI (per unit BW) induced by 48-h food deprivation (from 09.00 on day 1 until 09.00 on day 3). In control experiments PFS was used. Normally fed animals at ages 2, 4, 6, 12, 18 and 24 months, a group of CR animals (CR12 with unlimited access to powdered chow during the 3-h re-feeding), and two groups of HF rats (HF6 and HF12) were tested in the experiments.

Food intake-related alarin actions were tested in different settings: 1) alarin was given in the early phase of the inactive daytime period at 09:00 h and the 3-h and 24-h cumulative FI values were measured and compared with the effects of a NPY-injection as a positive orexigenic control; 2) alarin was injected at the onset of the active nighttime period at 18:00 h and 24-h cumulative FI was measured; 3) alarin effect was also tested on 24-h fasting-induced, 3-h and 24-h re-feeding FI, in this case alarin was given at the onset of the re-feeding period at 09:00 h.

4.6 Statistical analysis

All experimental groups contained at least 6-8 rats. One-way ANOVA or repeated-measures ANOVA tests with Tukey's post hoc test using SPSS for Windows 11.0 software were applied for the statistical analysis of the data and SigmaPlot for Windows version 11.0 was applied for regression analysis. Differences were accepted as statistically significant at the level of $p < 0.05$. Mean ± S.E.M. are indicated in all figures.

5. Results

5.1 Effects of CCK – a catabolic peptide

Summary of the experiment protocol:

CCK administration	Measured parameter	Animal groups		
IP CCK	FI	NF2, NF4, NF6, NF12, NF18, NF24	HF6, HF12	CR12
	Tc, Ts, VO_2, Ta	NF4		
ICV CCK	FI	NF2, NF4, NF6, NF12, NF24		
	Tc, Ts, VO_2, Ta	NF2, NF4, NF6, NF12, NF18, NF24		

Table 2. *Summary of experiments of intraperitoneally (IP) or intracerebroventricularly (ICV) applied CCK. Food intake (FI) and thermoregulatory parameters (core temperature – Tc; tail skin temperature – Ts; oxygen consumption – VO_2; ambient temperature – Ta) were measured in animals of different age-groups and nutritional states (NF =normally fed, HF = high fat, CR = caloric-restricted).*

5.1.1 Thermoregulatory and metabolic effect of CCK

For thermoregulatory analysis of CCK, different ambient temperatures were applied: thermoneutrality (25-28 ºC) allows the activation of vasodilation (heat loss), a cool environment (20 ºC) that elicits an increase in metabolic rate, permits the appearance/study of hypometabolic effects. On the graphicons full symbols represent changes following CCK-treatment, empty symbols represent controls [effects of pathogen-free saline (PFS) injection] respectively.

Young adult NF4 rats responded with hypothermia to IP injection of a pharmacological dose of CCK, in line with data of the literature (Kapas et al., 1987) due either to skin vasodilation (at a thermoneutral ambient temperature, Figure 7A) or to decrease in metabolic rate (at a cool ambient temperature, Figure 7B). Dependence of the hypothermic response on age or nutritional state was not analyzed in the present study because of technical reasons. In young adult NF4 rats the metabolic effects of ICV injected CCK appeared to be coordinated (Figure 7C), at thermoneutral ambient temperature. Centrally applied CCK caused an immediate significant rise in oxygen consumption with a decreasing tendency in RQ from 0.80 ± 0.01 to 0.76 ± 0.02 (suggesting a somewhat enhanced fat utilization) that did not reach statistical significance ($p = 0.061$, Figure 8). No change in tail vasomotor tone and Ts was caused by CCK at the lower end of the thermoneutral zone (25 ºC), i.e. the skin vasoconstriction persisted (Figure 7C). This response corresponds to the febrile reaction seen in response to lipopolysaccharide or PGE, and the anorexic effect fits the pattern of sickness behavior adjoining fever.

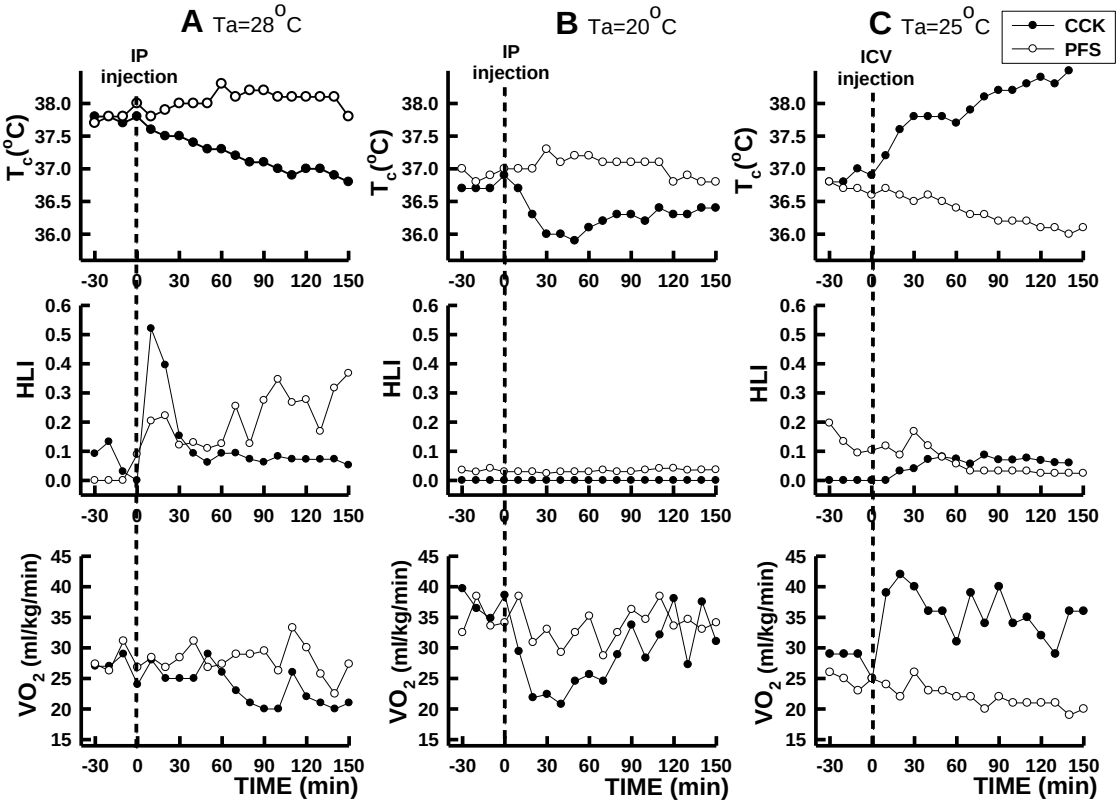

Figure 7. *Effects of IP or ICV CCK administration on metabolic rate and thermoregulation. The curves demonstrate representative individual recordings of core temperature (Tc), heat loss index (HLI) and oxygen consumption (VO_2) in a normally fed 4 months old rat at different ambient temperatures (Ta). Panel A: CCK was injected IP (100 μg) at an ambient temperature of 28 ºC. Panel B: CCK was injected IP (100 μg) at an ambient temperature of 20 ºC. Panel C: CCK was applied ICV (500 ng) at an ambient temperature of 25 ºC.*

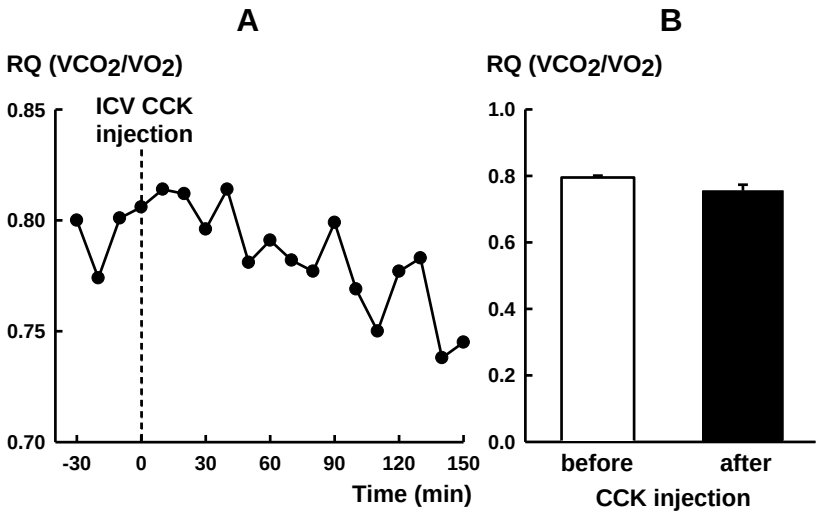

Figure 8. *Effect of intracerebroventricular (ICV) CCK administration on respiratory quotient (RQ). Panel A: The curve represents an individual recording of RQ upon CCK injection in a normally fed 4 months old rat. Panel B: RQ values expressed as mean ± S.E.M. for a group of normally fed 4 months old rats (n = 6) before and after (at 120 min) an ICV CCK injection. For statistical analysis one-way ANOVA test was used.*

The metabolic response to ICV CCK was age-dependent (Figure 9). The hypermetabolic/hyperthermic response that was characteristic for the NF4 rats became smaller, though still significant in NF6 and NF12 animals compared with the PFS-treated controls, but with further aging the response became even smaller and neither statistically nor biologically significant in the NF18 and NF24 old age-groups. Initial Tc values were similar in all groups (ranging from 37.4 ± 0.2 to 37.7 ±0.2 ºC).

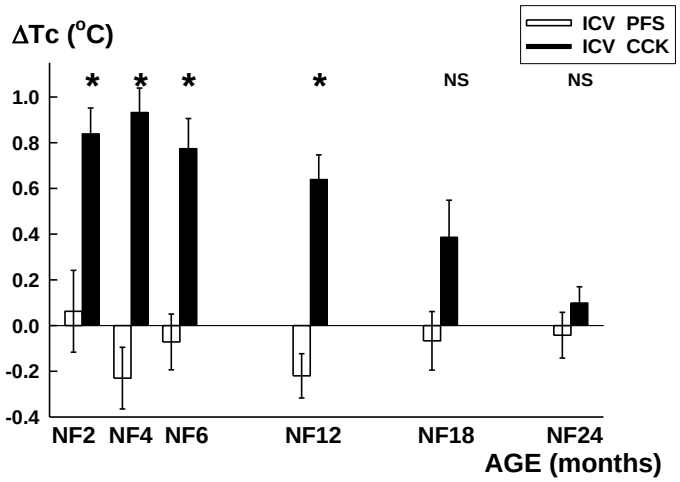

Figure 9. *CCK-induced hyperthermia in different age-groups of normally fed (NF) rats. Full columns represent changes in core temperature (ΔTc) at 120 min following an intracerebroventricular (ICV) CCK injection (500 ng), empty columns indicate similar values of controls following ICV PFS injections. Asterisks indicate significant differences between ΔTc of ICV CCK-treated and control rats of the same age. Each group contained 6-8 rats. For statistical analysis one-way ANOVA test was used.*

A negative linear correlation was shown between age and the CCK-induced change in Tc of individual rats (Figure 10). Correlation coefficient of the linear regression was r=-0648. These results suggested an age-related monotonous decrease in the hypermetabolic effect of centrally applied CCK.

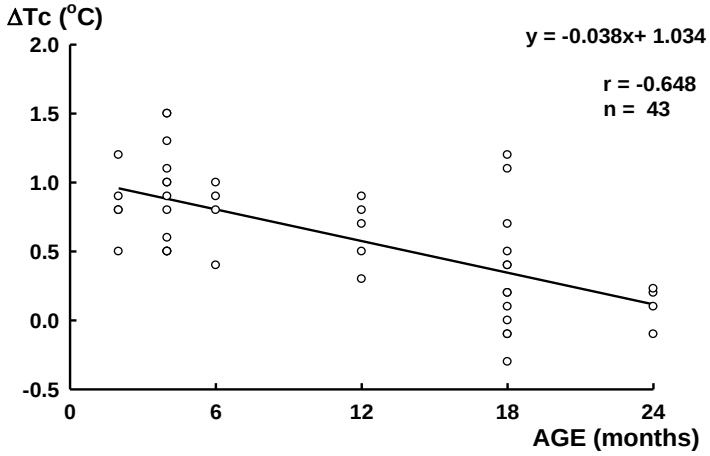

Figure 10. *Dependence of core temperature changes (ΔTc) induced by intracerebroventricular (ICV) CCK on age as shown by regression analysis. Empty symbols depict individual values of CCK-induced change in Tc of rats belonging to different age-groups (n = 43). One symbol may represent several identical values.*

5.1.2 Body weight and body composition of the groups of different age and nutritional state.

Body weight (Table 3) and body composition values (calculated for 100 g BW) of different NF age-groups (Figure 11 and Table 4) were in accord with those observed in our previous studies (Petervari et al., 2010): up to 12 months of age BW showed a rising tendency with a plateau phase between 12 and 18 months, then it started to decline slowly. Upon 48-h fasting weight loss of NF age-groups ranged from 7 % to 10% of initial BW except for a 14 % BW fall in the 2 months old juvenile group. Weight loss of CR12 rats reached about 10%, while HF animals lost merely 4-6 %.

Group	BW (g)	Group	BW (g)	Group	BW (g)
NF2 control	208.9 ± 5.6				
NF2 CCK	209.6 ± 1.6				
NF4 control	396.2 ± 12.2				
NF4 CCK	395.7 ± 11.4				
NF6 control	467.5 ± 14.6	HF6 control	565.0 ± 13.9*		
NF6 CCK	466.8 ± 12.6	HF6 CCK	546.6 ± 15.5*		
NF12 control	534.7 ± 10.4	HF12 control	710.1 ± 24.6#	CR12 control	324.4 ± 5.5*
NF12 CCK	529.1 ± 12.9	HF12 CCK	682.3 ± 23.5#	CR12 CCK	327.4 ± 5.7*
NF18 control	536.3 ± 17.2				
NF18 CCK	518.8 ± 7.2				
NF24 control	514.9 ±17.5				
NF24 CCK	511.8 ± 30.2				

Table 3. *Body weight values (BW) before a 48-h fasting of rats belonging to different age-groups and nutritional states (NF =normally fed, HF = high fat, CR = caloric-restricted). Concerning initial BW values the following statistically significant differences were denoted in the table: # HF12 vs. all other groups ($p<0.001$), * HF6 or CR12 vs. age-matched NF (HF6 vs. NF6 $p<0.01$, CR12 vs. NF12 $p<0.001$). Each group contained 6-8 rats. For statistical analysis one-way ANOVA test was used.*

In juvenile rats BW and fat mass indicators were significantly smaller than those of all other groups. No difference in muscle mass was detected in any group, except for the oldest (24 months old) sarcopenic animals. Body weight (Table 3) and fat content of HF rats exceeded those of age-matched NF controls (Figure 11), while the relative muscle mass remained similar (Table 4).

Group	Muscle indicator (g/100g BW)
NF2	0.18 ± 0.01
NF4	0.17 ± 0.01
NF6	0.20 ± 0.01
HF6	0.19 ± 0.01
NF12	0.19 ± 0.01
HF12	0.18 ± 0.01
CR12	0.20 ± 0.01
NF18	0.17 ± 0.01
NF24	0.13 ± 0.01*

Table 4. *Indicator of muscle mass in rats of different age-groups and nutritional states (NF =normally fed, HF = high fat, CR = caloric-restricted). Each group contained 6-8 rats. For statistical analysis one-way ANOVA test was used.*

Body composition indicators of NF6 vs. HF6 rats were as follows: epididymal fat: 0.35 ± 0.04 vs. 0.77± 0.07 g/100g BW ($p < 0.001$); retroperitoneal fat: 0.39 ± 0.07 vs. 1.07 ± 0.15 g/100g BW ($p = 0.002$). Similar ratios were observed in the NF12 vs. HF12 groups: epididymal fat: 0.52 ± 0.04 vs. 1.00 ± 0.05 g/100g BW ($p < 0.001$); retroperitoneal fat: 0.50 ± 0.03 vs. 1.82 ± 0.19 g/100g BW ($p < 0.001$). Fat mass and BW values of HF12 were significantly higher than those of all other rats. Although HF6 weighed less than HF12, their BW was comparable to (or even higher than that of) NF12, twice their age (Table 3).

Body weight (Table 3) and fat content (Figure 11) of CR12 rats were significantly smaller than those of age-matched NF controls. These values were similar to those of much younger NF4 animals. While fat mass was significantly reduced, no decline of muscle mass was observed compared to NF12 (Table 4): epididymal fat: 0.52 ± 0.04 vs. 0.26 ± 0.03 g/100g BW ($p = 0.002$); retroperitoneal fat: 0.50 ± 0.03 vs. 0.14 ± 0.03 g/100g BW ($p < 0.001$) (NF12 vs. CR12, respectively).

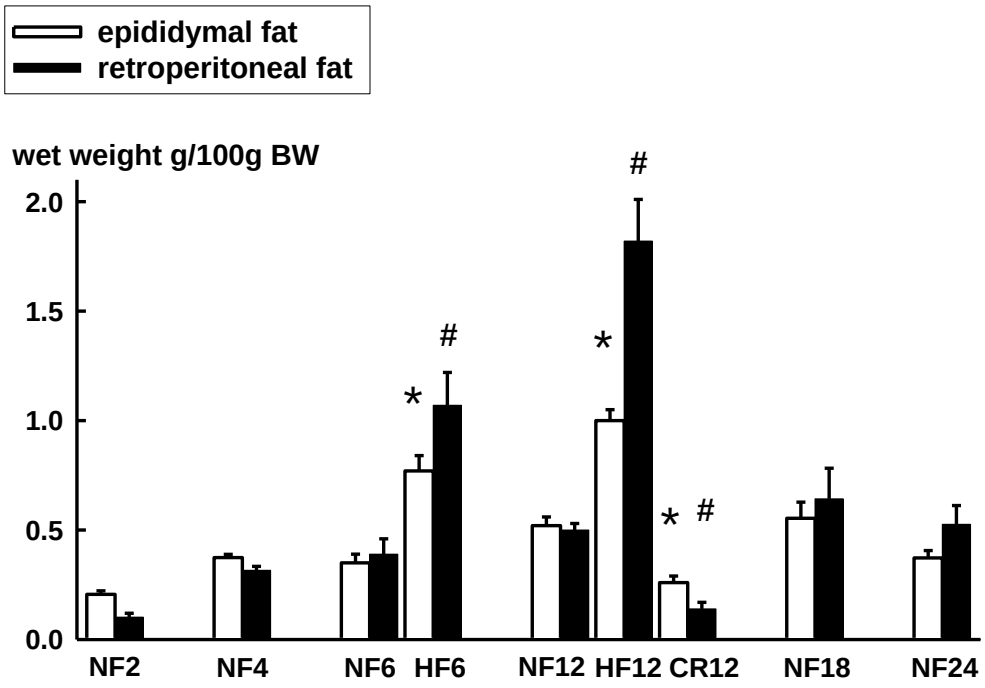

Figure 11. *Indicators of fat mass in rats of different age-groups and nutritional states (NF = normally fed, HF = high fat, CR = caloric-restricted) given for 100 g body weight. For statistical analysis one-way ANOVA test with Tukey post-hoc test was used. Asterisks indicate significant difference of epididymal fat as compared with the corresponding NF age-group. Each group contained 6-8 rats. Number signs indicate significant difference of epididymal fat as compared with the corresponding NF age-group.*

5.1.3 Effect of CCK on food intake

The cumulative energy intake during 3-h re-feeding of rats belonging to different age-groups and nutritional states are showed in Table 5. Re-feeding expressed in kJ was largest in the HF animals, values of HF6 exceeded those of HF12 (Table 5). When calculated for 100 g BW, 3-h energy intake of HF6 was circa twice as high as that of HF12 (40.7 ± 4.7 vs. 22.5 ± 2.0 kJ/100g BW, Figure 13). Regarding CR12 rats, their cumulative 3-h energy intake in kJ-s following 48-h fasting was also very high: it exceeded significantly the value of NF12, but did not differ from that of HF12. When expressed in kJ/100 g BW re-feeding energy intake of CR12 was the largest of all rats (47.8 ± 4.2 kJ/100 g BW, Figure 13).

Group	FI (kJ)	Group	FI (kJ)	Group	FI (kJ)
NF2 control	78.4 ± 6.8				
NF2 CCK	72.3 ± 6.2				
NF4 control	134.1 ± 5.8				
NF4 CCK	99.0 ± 6.9[c]				
NF6 control	137.5 ± 17.2	HF6 control	215.6 ± 24.3[a]		
NF6 CCK	89.7 ± 7.6[c]	HF6 CCK	181.9 ± 31.1		
NF12 control	86.9 ± 3.2	HF12 control	153.4 ± 13.9[b]	CR12 control	139.7 ± 11.7[b]
NF12 CCK	78.1 ± 5.6	HF12 CCK	98.7 ± 13.5[c]	CR12 CCK	73.9 ± 9.8[c]
NF18 control	102.7 ± 10.7				
NF18 CCK	71.5 ± 5.8[c]				
NF24 control	102.7 ± 7.7				
NF24 CCK	64.9 ± 6.6[c]				

Table 5. *Cumulative energy intake (FI) during the 3-h re-feeding of rats belonging to different age-groups and nutritional states Regarding 3-h cumulative FI values of control groups the following statistically significant differences were denoted in the table: "a" HF6 vs. all other groups ($p<0.05$), "b" HF12 or CR12 vs. age-matched NF (HF12 vs. NF12 $p<0.001$, CR12 vs. NF12 $p<0.01$). Each group contained 6-8 rats. For statistical analysis one-way ANOVA test was used.*

In young adult rats the IP administered CCK caused significant suppression of 3-h cumulative food intake during re-feeding after 48-h fasting. In order to demonstrate the different rates of suppression Figure 12 shows these data in kJ/100 g BW. In NF rats, the subsequent cumulative 3-h energy intake (during re-feeding) showed an age-dependent decline from NF2 to NF12 and remained at the same level thereafter. Compared to the suppression seen in the young adult group, the most pronounced effect was observed at the age of 6 months. However, CCK was ineffective in juvenile animals and in middle-aged ones (NF12). Interestingly, at later ages (NF18, NF24) the suppression of re-feeding energy intake became again pronounced and statistically significant.

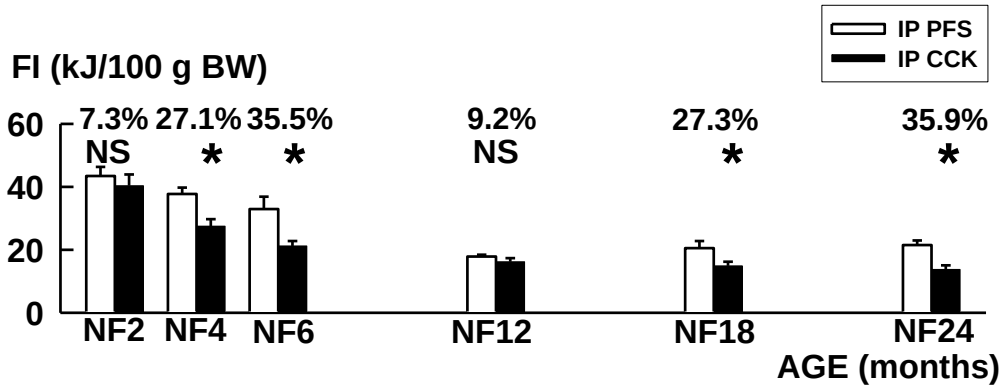

Figure 12. *Reduction in the 48-h fasting-induced cumulative 3-h FI expressed in kJ/100 g BW, (after fasting, before re-feeding) in different age-groups and nutritional states of rats following intraperitoneal (IP) CCK treatment. The rate of reduction is denoted above the pairs of columns in percentage of the corresponding control value. Asterisks indicate significant differences between re-feeding values of IP CCK-treated (dark columns) and control (PFS-treated) rats of the same age and nutritional state (light columns). Each group contained 7-15 rats. For statistical analysis one-way ANOVA test was used.*

Regarding the anorexic efficacy of CCK in different age groups, the highest relative dose normalized to BW (Table 6) failed to elicit significant effects in NF2, proceeding to exert reduction in FI despite lower relative CCK doses in NF4 and NF6. Although middle-aged rats were characterized by a low relative dose with lack of CCK-anorexia, the same low relative dose proved to be efficient from NF18.

Group:	**NF2**	**NF4**	**NF6**	**NF12**	**NF18**	**NF24**
Dose normalized to BW (μg/100 g BW)	2.4	1.3	1.1	0.9	0.9	1.0
Group:			**HF6**	**HF12**		
Dose normalized to BW (μg/100 g BW)			0.9	0.7		
Group:				**CR12**		
Dose normalized to BW (μg/100 g BW)				1.5		

Table 6. *Dose of 5 μg CCK normalized to body weight (BW) in animals belonging to different age groups and nutritional states (normally fed=NF, HF= high fat, CR= caloric-restricted).*

Alterations in body composition influenced this pattern of CCK efficacy. Obese HF6 rats (with low relative dose, see Table 6) were resistant to the anorexic effect of CCK (Figure 13) already at the age of 6 months, but the anorexic effect became again significant in HF12 animals (lowest relative dose). In contrast, CR12 rats consumed during re-feeding much more food than age-matched controls (they appeared to be hungrier than rats of the NF12 group), but CCK almost halved the consumption (Figure 13), unlike in NF12 animals.

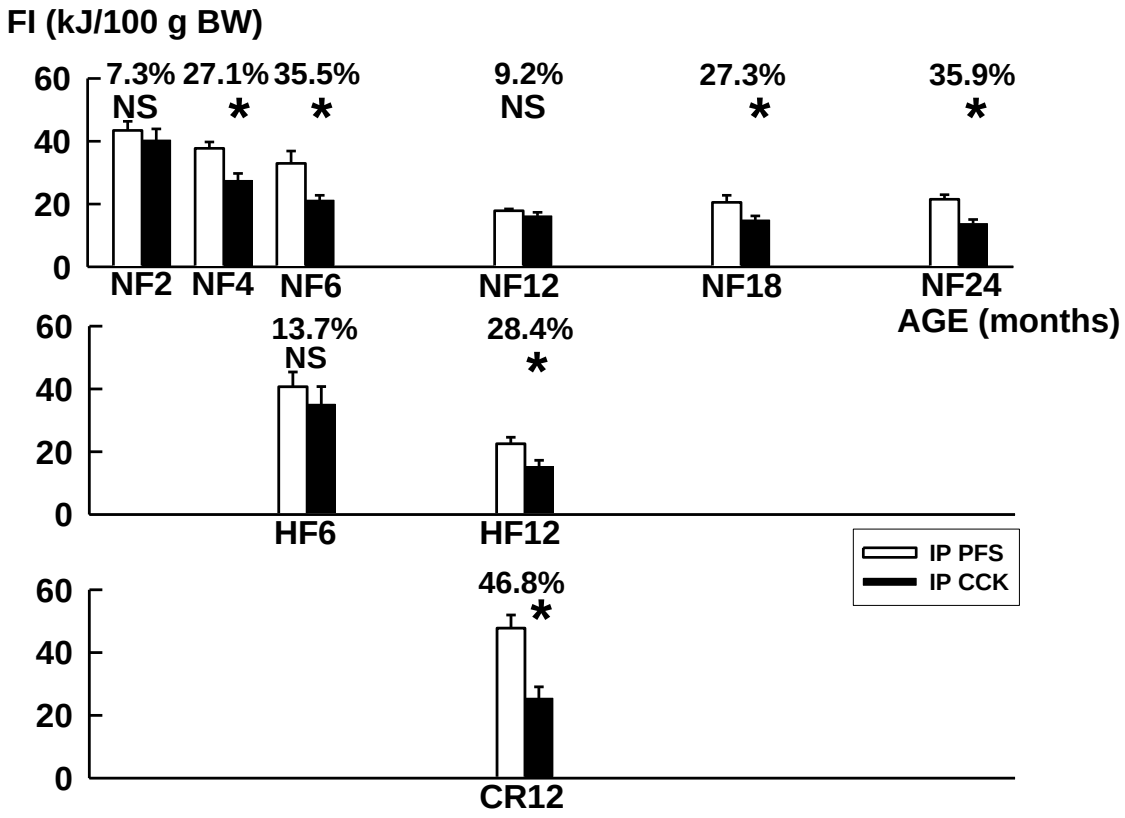

Figure 13. *Reduction in the 48-h fasting-induced cumulative 3-h food intake (FI) expressed in kJ/100 g BW, (after fasting, before re-feeding) in different age-groups and nutritional states (NF =normally fed, HF = high fat, CR = caloric-restricted) of rats following intraperitoneal (IP) CCK treatment. Each group contained 7-15 rats. For statistical analysis one-way ANOVA test was used.*

The ICV injected CCK (Figure 14) was also without significant effect on food intake in juvenile rats, but caused extreme anorexia in NF4 and NF6 animals (peak suppression in NF6). The effect was attenuated but still significant in the middle-aged NF12 group, and – in contrast to the IP administration – it further decreased and became non-significant in the old NF24 animals similarly as seen in case of the age-related decline in the metabolic effect of ICV CCK.

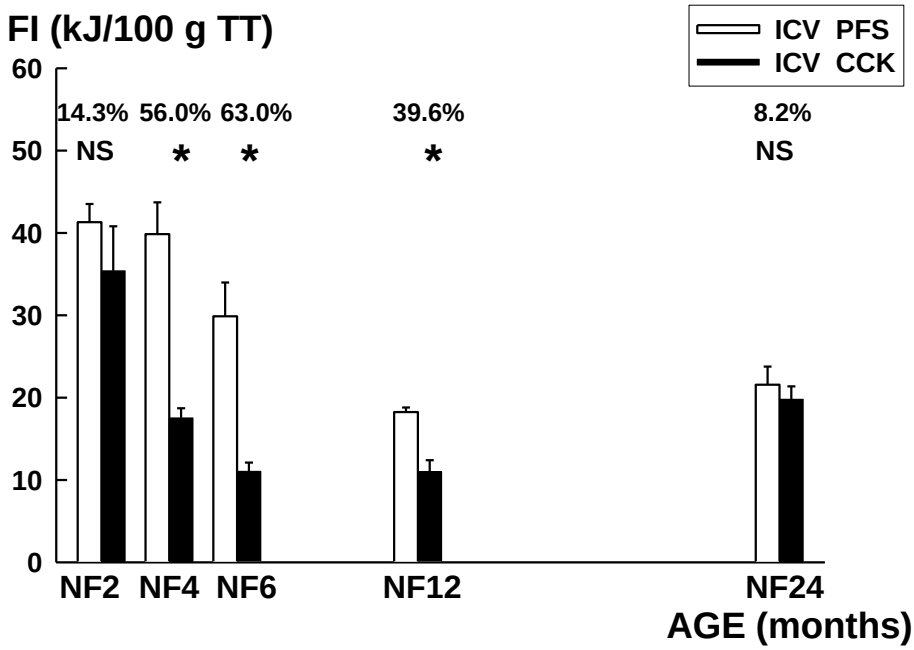

Figure 14. *Reduction in the 48-h fasting-induced cumulative 3-h food intake (FI) expressed in kJ/100 g BW after fasting, before re-feeding in different age-groups of normally fed (NF) rats following intracerebroventricular (ICV) CCK treatment. The rate of reduction is denoted above the pairs of columns in percentage of the corresponding control value. Asterisks indicate significant differences between re-feeding of ICV CCK-treated (dark columns) and control (PFS-treated) rats of the same age. Each group contained 6-8 rats. For statistical analysis one-way ANOVA test was used.*

5.2 Effects of alarin – a catabolic peptide

5.2.1 Thermoregulatory effects of alarin

An acute ICV injection of 3 μg full-length alarin elicited a delayed significant increase in VO_2 at 25 ºC. It is demonstrated in percentage of the initial value (21.42 ± 1.84 vs. 22.01 ± 1.35 ml/kg/min for 3 vs. 0 μg alarin) ($F(1,13) = 21.585$, $p < 0.001$) (Figure 15). This hypermetabolism and concurrent continuous tail skin vasoconstriction, as shown by the low value of the HLI, induced a slow but significant rise in Tc that reached 0.5 ºC by 120, and 1.0 ºC by 180 minutes after the injection ($F(1,13) = 28.005$, $p < 0.001$). HLI was calculated according to the literature (Romanovsky et al., 2000), ranges between 0 and 1. As the value approaches 0 – vasoconstriction, when the value approaches 1 − vasodilatation is indicated.

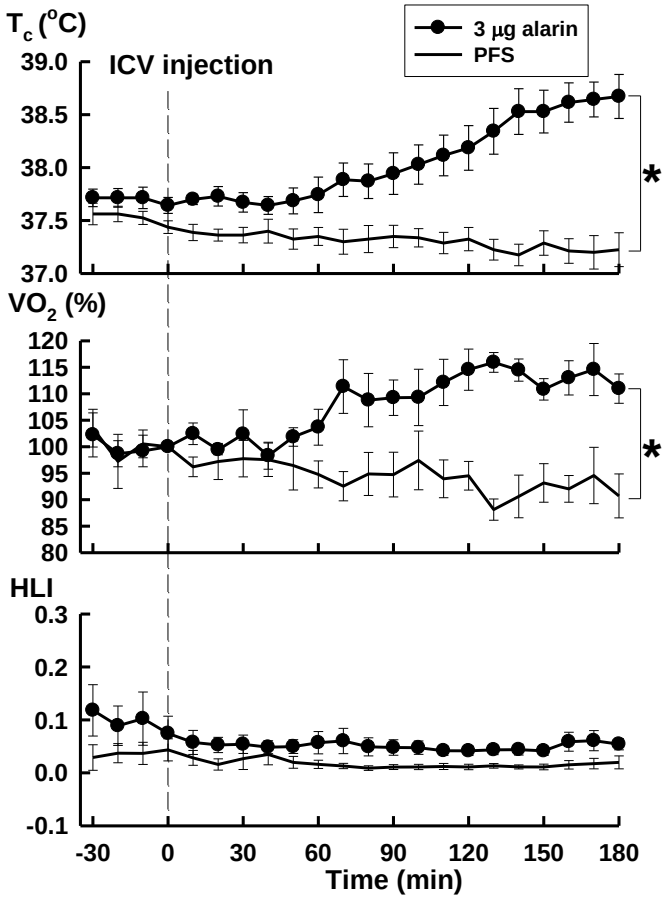

Figure 15. *Changes of core temperature (Tc), heat loss index (HLI) and oxygen consumption (VO_2) following an ICV injection of 3 μg alarin (n = 7) or pyrogen-free saline (PFS) (n = 8) in young adult male Wistar rats at 25°C ambient temperature. For statistical analysis repeated-measures ANOVA test was used. Asterisks indicate significant differences.*

Different doses of alarin (1, 3 or 15 μg) induced significant hyperthermias at 25 ºC (Figure 16A), i.e. no dose-dependence was observed. For clarity, the dose of 0.3 μg is not shown. The initial Tc values ranged from 37.38 to 37.64 °C, the mean initial Tc values were similar in all groups. The hyperthermic response was significant for each dose as compared with the effects of scrambled alarin [1 μg: $F(1,11) = 20.483$, $p = 0.001$; 3μg: $F(1,12) = 6.428$, $p = 0.026$; 15 μg: $F(1,8) = 9.567$, $p = 0.015$]. Scrambled alarin failed to induce any response (Figure 16A). At cooler ambient temperatures [15 ºC (Figure 16B) or 20 ºC (not shown)] the administration of 3 μg alarin resulted in similar Tc rises as those seen at 25 ºC. Initial core temperatures ranged from 37.42 to 37.70 °C. [For 25 °C 0 vs. 3 μg: $F(1,12) = 27.454$, $p < 0.001$; for 15 °C $F(1,8) = 13.731$, $p = 0.006$].

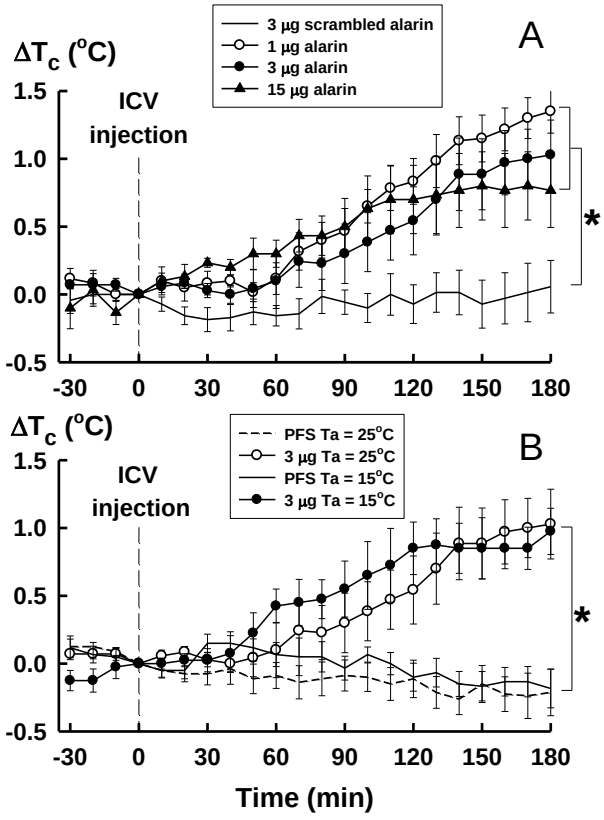

Figure 16. *Upper panel: Changes in core temperature (ΔTc) upon ICV administration of different doses of alarin in young adult male Wistar rats at 25 °C ambient temperature as compared with the respective initial values. Lower panel: Changes in core temperature (ΔTc) upon ICV administration of 3 μg alarin or pyrogen-free saline (PFS) at 15 or 25 °C ambient temperatures. For statistical analysis repeated-measures ANOVA test was used. Asterisk indicates on the panel A significant difference of groups treated with different doses (1, 3 or 15 μg) of alarin as compared with the scrambled alarin-treated group. All four groups contained 6-7 rats. Asterisk indicates on the panel B significant difference of 3 μg alarin treated group as compared with the PFS-treated group at the same ambient temperature. All four groups contained 6-7 rats.*

Around the vasodilation threshold (28 ºC) a pronounced alarin-induced vasoconstriction was accompanied by a significant hyperthermic effect as shown in the individual recording of Figure 17. Upon peripheral injections, full-length alarin failed to induce hyperthermia or vasoconstriction (not shown).

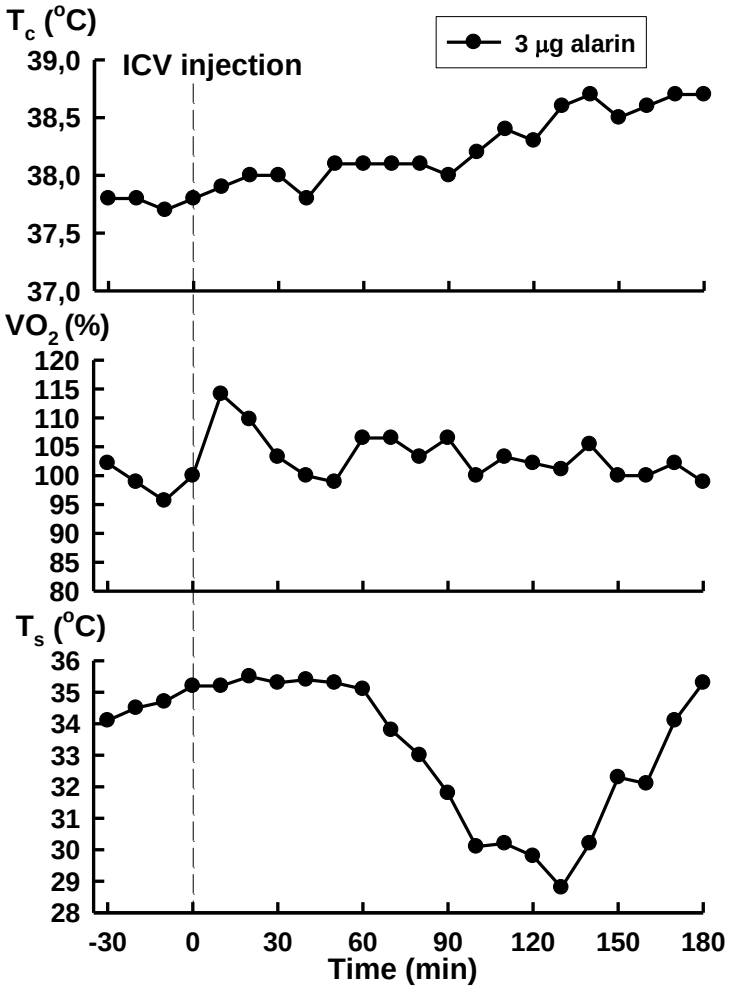

Figure 17. *Representative individual recording of the rise in core temperature (Tc) (upper panel), in oxygen consumption (VO_2) in percent of the initial value (middle panel, initial value 20.75 ml/kg/min) and the fall in tail skin temperature (Ts) indicating vasoconstriction (lower panel) upon intracerebroventricular (ICV) injection of 3 μg alarin at 28 °C ambient temperature.*

According to earlier data (Fraley et al., 2012), elimination of the first 5 amino acids of alarin (alarin 6-25Cys, 2.5 μg) abolished the orexigenic action, i.e. resulted in an antagonist effect. In our experiments, upon central administration truncated alarin did not elicit any significant thermoregulatory response.

Truncated alarin, when administered together with ICV full-length alarin (3 μg), abolished the hyperthermic action of full-length alarin (Figure 18). Thus an antagonistic effect of alarin 6-25Cys was demonstrated. The hyperthermic effect of full-length alarin significantly differed from that of the truncated form ($F(1,13) = 14.550$, $p = 0.002$) or from that of the combined administration ($F(1,14) = 7.624$, $p = 0.015$). Initial core temperatures ranged from 37.42 to 37.65 °C and were similar in all groups.

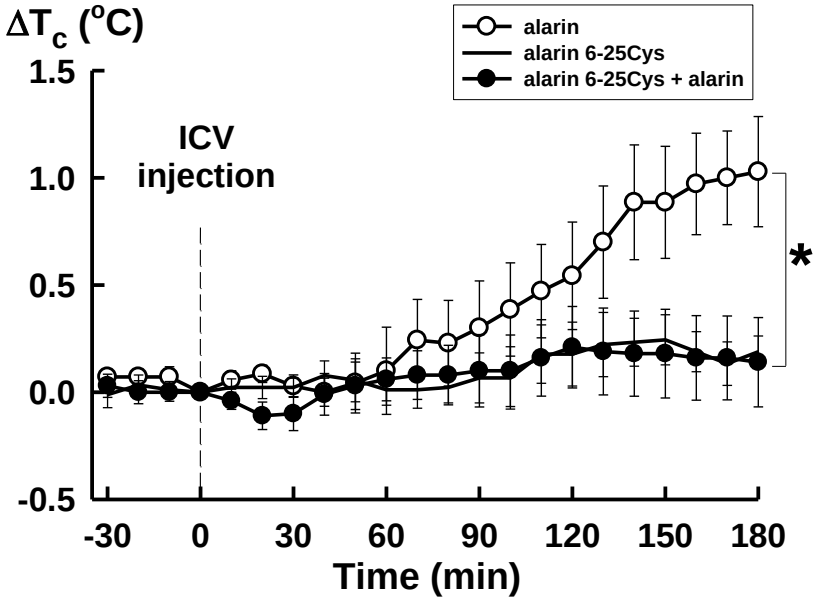

Figure 18. *Changes in core temperature (ΔTc) upon intracerebroventricular (ICV) administrations of alarin, truncated alarin (Ala6-25Cys) or an alarin + Ala6-25Cys combination in young adult male Wistar rats at 25 °C ambient temperature. All three groups contained 7-10 rats. For statistical analysis repeated-measures ANOVA test was used. Asterisk indicates significant difference of alarin-treated group as compared with other two groups treated with Ala6-25Cys.*

Acute ICV injection of alarin (1, 3 or 15 μg) induced a significant increase in VO_2 as compared to vehicle treated animals ($p < 0.001$, $p = 0.006$,$p = 0.049$) at 25 ºC (Figure 19, upper panel). This hypermetabolism was associated by a simultaneous tail skin vasoconstriction (heat conservation). These coordinated changes resulted in a significant fever-like rise in Tc that exceeded 1.0 ºC by 180 min post-injection [Figure 19, lower panel (compared to the vehicle group: $p < 0.001$ in case of all doses,)]. Even this high increase in Tc failed to elicit any compensatory vasodilation. As all doses of alarin induced similar hypermetabolism and hyperthermia, no dose-dependence was observable. We investigated the possible prostaglandin-mediation in this fever-like hyperthermia. The effects of COX inhibitors were tested on the hyperthermic/hypermetabolic response to 3 μg alarin. Neither applied COX inhibitor *per se* induced any thermoregulatory response (Figure 19, upper and lower panel). Systemic pre-treatment of indomethacin (2 mg/kg, IP), a non-specific COX-inhibitor given 30 min before the ICV injection prevented alarin-induced hypermetabolism ($p < 0.001$) and hyperthermia ($p = 0.017$). Both applied doses of meloxicam, a relatively selective COX-2 inhibitor, given 30 min prior to the ICV injection effectively reduced hypermetabolic ($p < 0.001$ for both doses) and hyperthermic ($p = 0.049$ for 1 mg/kg, $p = 0.028$ for 2 mg/kg) responses to alarin. Such a thermoregulatory effect would rather characterize a catabolic mediator and not an anabolic one, although previous studies reported some orexigenic effect of the peptide.

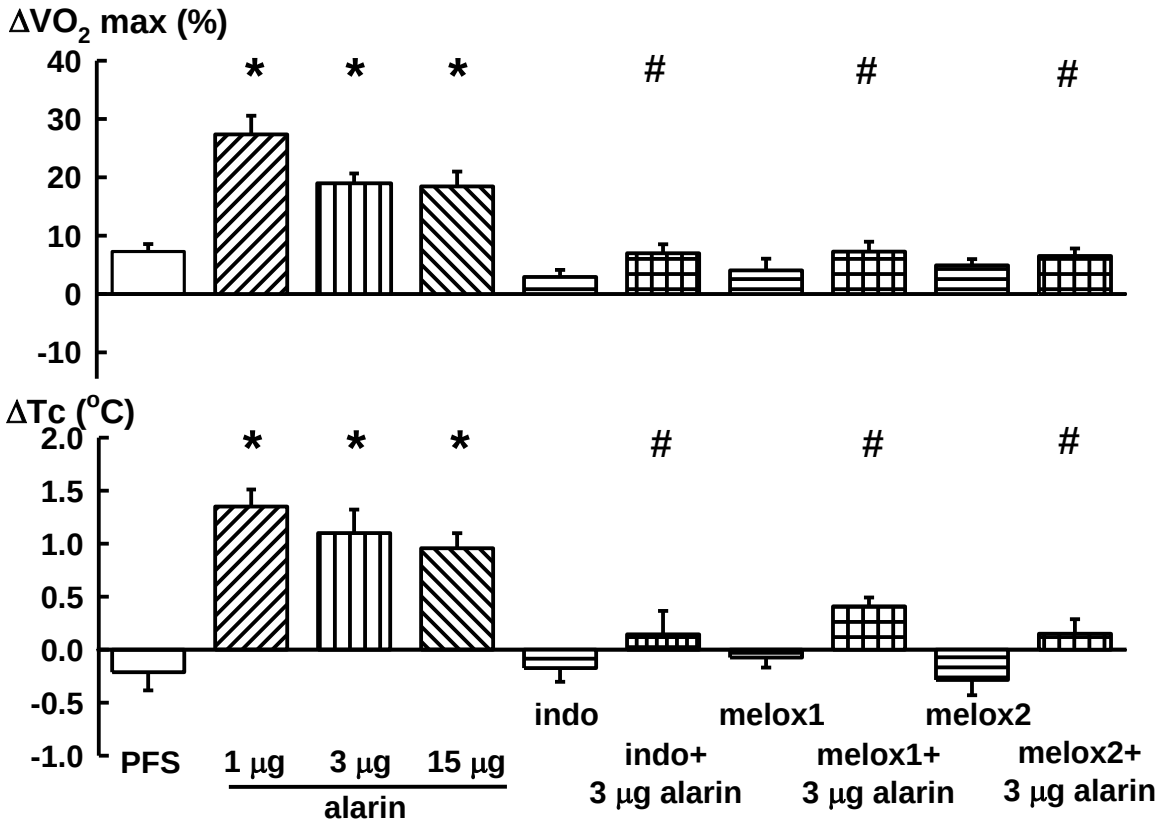

Figure 19. *Hypermetabolic and hyperthermic effects of alarin (1, 3 and 15 μg) or pyrogen-free saline (PFS). The upper panel shows maximal increase in oxygen consumption in % of the corresponding initial value (ΔVO₂max). The lower panel demonstrates the increase in core temperature as compared to the corresponding initial value, at 180 min after the ICV injection (ΔTc). Both effects of 3 μg alarin were significantly attenuated by pretreatment with intraperitoneal (cyclooxygenase) COX inhibitors: indomethacin (non-selective COX inhibitor, 2 mg/kg) and meloxicam (relatively selective COX-2 inhibitor, 1 or 2 mg/kg). Each group contained 6-9 rats. For statistical analysis one-way ANOVA test was used. Asterisks (*) indicate significant differences from the vehicle treated control group (PFS); number signs (#) indicate significant differences from the effect of 3 μg alarin. Initial VO_2 (21.47 ± 1.89 ml/kg/min) and Tc (37.50 ± 0.15°C) values were similar in all groups.*

Individual record of Tc demonstrates the inhibitory effect of IP injection of meloxicam (1 mg/kg) on hyperthermia induced by ICV alarin injection (3 μg) (Figure 20). The correct ICV administration was confirmed by prostaglandin E1 (PGE1, 100 ng) injection at 180 min, that was able to induce prompt hyperthermic reaction.

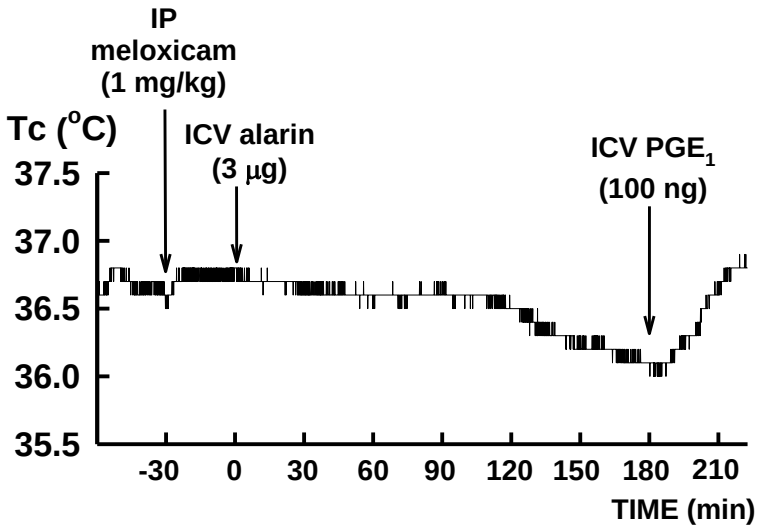

Figure 20. *Representative individual recording of Tc demonstrates the inhibitory effect of intraperitoneal (IP) injection of meloxicam (1 mg/kg) on hyperthermia induced by intracerebroventricular (ICV) alarin injection (3 μg). The correct ICV administration was confirmed by PGE_1 (100 ng) injection at 180 min, that was able to induce prompt hyperthermic reaction.*

5.2.2 Effects of alarin on food intake

Initial BW of 3 months old young adult Wistar rats was 340 ± 20 g. We started to test the potential orexigenic effects of alarin as compared with those of NPY. Alarin (1 or 3 μg) given at 09:00 h (in the early phase of the inactive daytime period) failed to induce FI in non-deprived rats (Figure 21). In contrast, NPY (5 μg, as positive orexigenic control) was able to induce FI [$F(3,51) = 49.341$; NPY vs. other groups: $p < 0.001$] in the same animal group (Figure 21). However, cumulative 24-h FI following alarin administration (at 3 μg, but not 1 μg dose) was found to be suppressed (alarin versus control: 15.1 ± 1.4 g versus 21.9 ± 0.8 g, $p < 0.001$). Similarly, a central injection of NPY also reduced cumulative 24-h FI (NPY versus control: 16.8 ± 1.8 g versus 21.9 ± 0.8 g, $p = 0.010$) after its short-term orexigenic action. As these tests indicated a surprising anorexigenic action of alarin, we decided to test it in other settings.

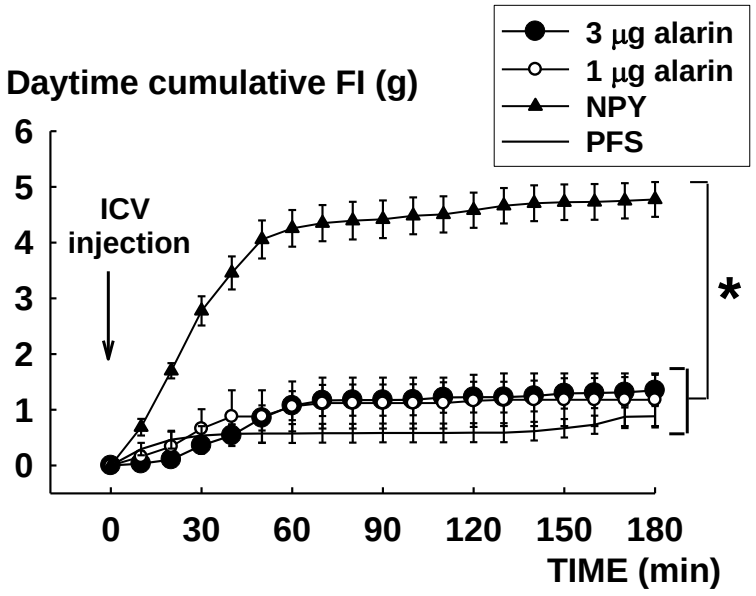

Figure 21. *In contrast to neuropeptide Y (NPY; 5 μg) ICV injection of alarin (1 or 3 μg) or pyrogen-free saline (PFS) failed to induce food intake (FI) during the daytime period. All four groups contained 6-7 rats. For statistical analysis repeated-measures ANOVA test was used. Asterisk indicates significant difference of groups treated with 1, 3 μg of alarin or PFS as compared with the NPY-treated (n = 7) group.*

Alarin (3 μg) given at 18:00 h (at the onset of the active nighttime period) strongly reduced spontaneous nighttime cumulative FI without any compensation in the following daytime period [for 24-h cumulative FI: $F(1,15) = 65.888$, $p < 0.001$] in non-deprived rats (Figure 22).

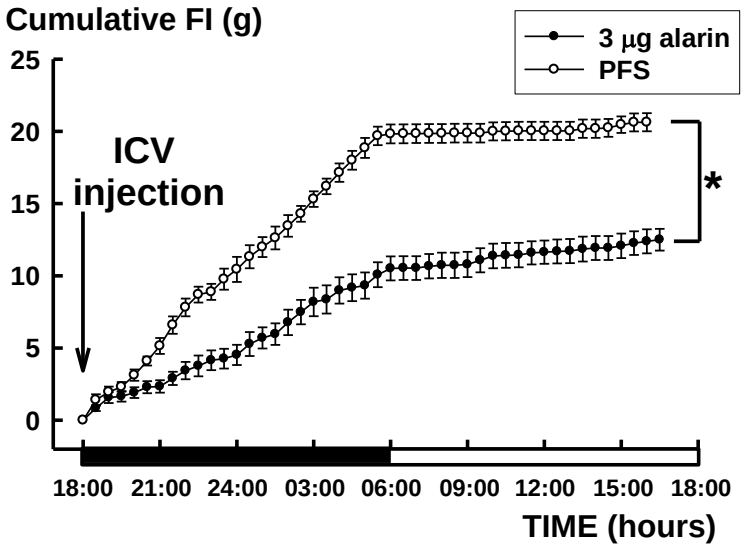

Figure 22. *Intracerebroventricular (ICV) alarin injection (3 μg) given at the onset of the nighttime period (18:00 h), significantly reduced cumulative spontaneous food intake (FI) (without any compensation in the following daytime period). Horizontal black bar underneath the abscissa refers to the dark period. Both groups contained 6 rats. For statistical analysis repeated-measures ANOVA test was used. Asterisk indicates significant difference.*

Finally, alarin (3 μg) was given at 09:00 h to 24-h fasted rats at the onset of their 180-min re-feeding. Upon 24-h fasting, their weight loss was 8.4 ± 0.4% of initial BW (BW before fasting: 355.1 ± 5.8 g, before re-feeding: 325.3 ± 5.3 g, initial BW of treated and those of control animals did not differ). The peptide suppressed re-feeding FI efficiently [for 180-min cumulative FI: $F(1,16) = 9.353$, $p = 0.008$] (Figure 23). In addition, this anorexigenic effect also proved to be long-lasting, as shown by the significant suppression of 24-h FI of these animals (alarin versus vehicle: 24.4 ± 1.5 g versus 29.5 ± 1.0 g, $p = 0.009$,).

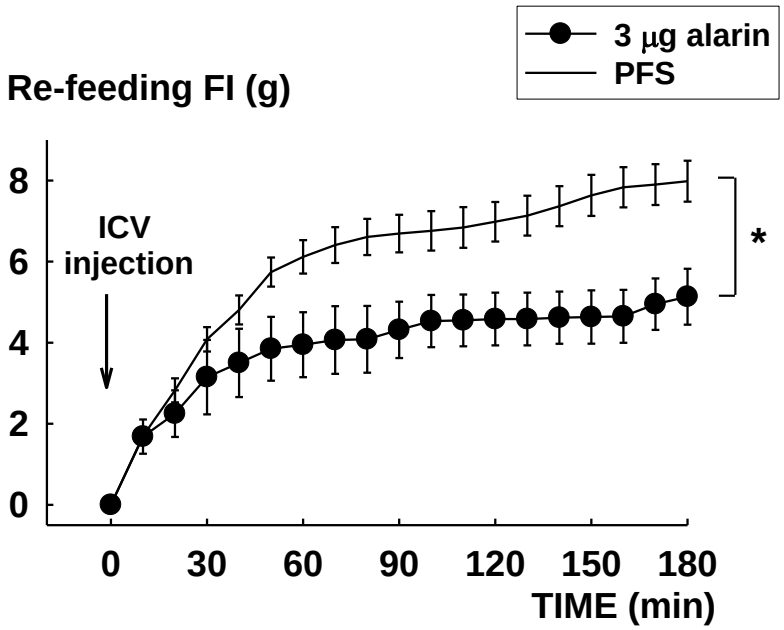

Figure 23. *Effect of intracerebroventricular (ICV) alarin injection (3 μg) or pyrogen-free saline (PFS) on24-h fasting-induced food intake (FI) during the daytime period. Both groups contained 6-9 rats. For statistical analysis repeated-measures ANOVA test was used. Asterisk indicates significant difference.*

Our results diverged from those of the literature. As those studies used younger (6 weeks old) Long-Evans rats (Van Der Kolk et al., 2010), we tested FI-associated effects of alarin in Wistar and Long-Evans rats of similar young age to investigate potential causes of these differences. A lack of orexigenic effects of alarin (0.3 and 3 μg) was demonstrated in both strains (data are given as supplementary information: Figure 24). NPY significantly enhanced FI both in Wistar rats [$F(3,19) = 36.600$; NPY vs. other groups: $p < 0.001$, repeated-measures ANOVA with Tukey's post hoc test] and in Long-Evans rats [$F(3,18) = 163.285$; NPY vs. other groups: $p < 0.001$].

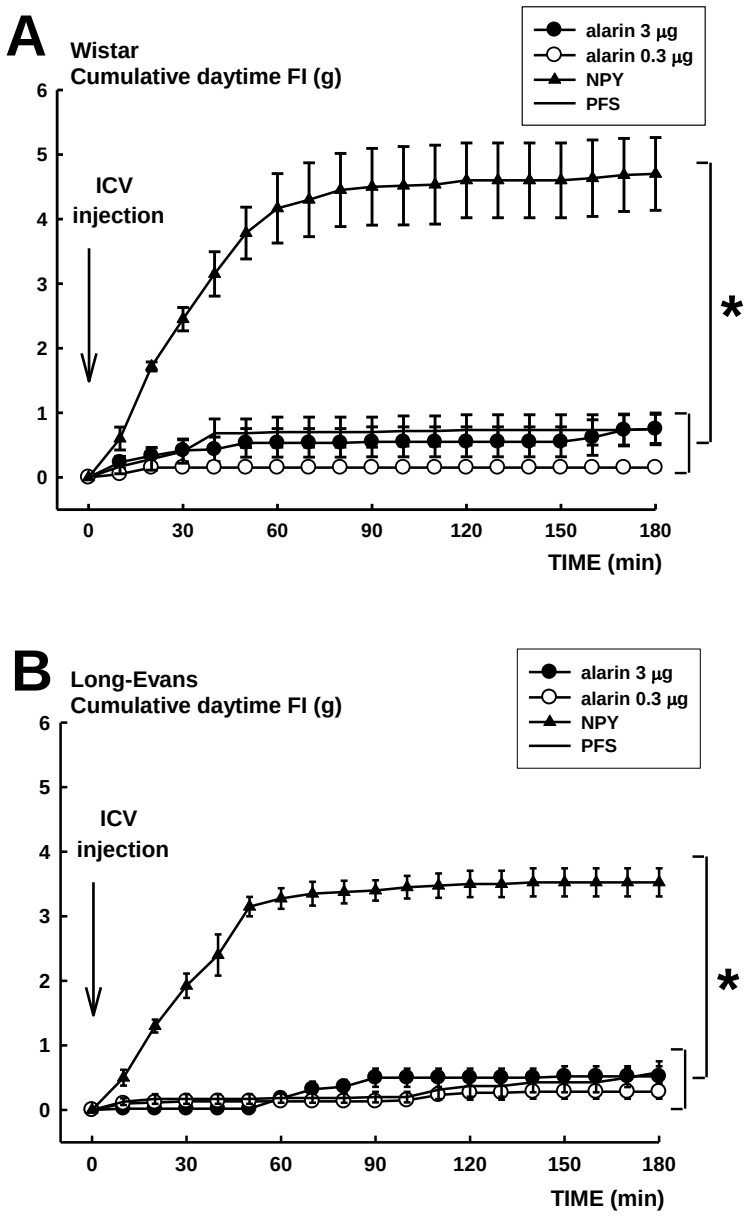

Figure 24. *In contrast to (NPY, 5 μg), ICV injection of alarin (0.3 or 3 μg) or pyrogen-free saline (PFS) failed to induce food intake (FI) during the daytime period in 6 weeks old male Wistar (panel A) or Long-Evans (panel B) rats. For statistical analysis repeated-measures ANOVA test was used. Asterisk indicates on panel A significant difference of groups treated with 0.3, 3 μg of alarin or PFS as compared with the NPY-treated group in Wistar rats All four groups contained 6-7 rats. Asterisk indicates on panel B significant difference of groups treated with 0.3, 3 μg of alarin or PFS as compared with the NPY-treated group in Long-Evans rats. All four groups contained 5-7 rats.*

6. Discussion and conclusions

6.1 CCK

Body weight development of male Wistar rats showed a steady increase until the age of 18 months followed by a decline in the oldest 24-month old age-group. In CCK-related experiments the high-fat diet induced significant weight gain and an accumulation of fat as shown by the body composition indicators. Body weight and fat mass indicators of the obese 6-month old rats exceeded those of normally fed older middle-aged 12-month old animals suggesting premature onset of „middle-aged" obesity induced by a high-fat diet. On the other hand, these parameters of caloric-restricted representatives of the 12-month age-group were significantly smaller than those of their normally fed controls. Moreover, these parameters were reduced below those of much younger normally fed 4-month old animals. This suggests an efficient prevention of „middle-aged" obesity by the applied level of caloric restriction.

Our results regarding the peripheral administration of CCK show that aging did not cause a gradual continuous decline in the efficacy of the peptide, rather age-related phasic changes were demonstrated for the anorexigenic CCK effect.

Whether given IP or ICV, in young adult NF4 and young middle-aged NF6 rats CCK significantly suppressed food intake (although the ICV applied 500 ng dose exerted stronger anorexigenic response in young adult rats than the IP injected 5 μg). Interestingly, both types of administration were ineffective in juvenile NF2 animals, suggesting the presence of an extremely strong orexigenic tone at this age. In juvenile rats similar "resistance" was demonstrated for the anorexic effect alpha-MSH – this was also explained by a high orexigenic tone, that is specific for this age of fast growth (Petervari et al., 2010).

The anorexic effect of IP CCK observed in young adult rats is in line with data of the literature (Smith and Gibbs, 1998, Balasko et al., 2012). Signals representing information from stretch of the stomach and from the nutrient composition of its content are conveyed by fibers of the abdominal afferent vagus (South and Ritter, 1988, Blessing, 1997, Berthoud, 2004) to the NTS, brainstem, and further structures of the brain. The hindbrain alone is sufficient for the development of such CCK-anorexia: peripheral CCK causes satiety even in decerebrate animals (Grill and Smith, 1988), but not in animals with NTS lesion (Edwards et al., 1986). Some of the vagal afferent fibers (C-type) contain CCK1R-s and they are capsaicin sensitive, while other (A-type) fibers

are insensitive. In rats, systemic capsaicin desensitization prevented the satiety induced by IP CCK (South and Ritter, 1988). However, decerebrate animals can only adapt to the short-term feeding state but not to long-term changes in nutrition (starvation, overfeeding). If CCK can indeed influence the long-term changes of energy balance, or such changes of nutritional state (starvation, obesity) can interfere with the effects of peripheral CCK, then other additional point(s) of action must be assumed. In fact, circulating CCK may also act at the ARC by enhancing the transport of (anorexic) leptin through the blood-brain-barrier (Cano et al., 2008), or possibly it acts directly at other structures of the brain. The anorexic effect of IP CCK in young adults appears to be related to the actual feeding state rather than the more chronic nutritional state, still, in rats the lack of CCK1R-s is connected with obesity [Otsuka Long Evans Tokushima (OLETF) rats], suggesting a possible long-term role of CCK1R activity in energy balance. These rats eat more and become obese, probably due to lack of satiety and to a high hypothalamic NPY tone (Bi et al., 2007). Satiety deficit has also been demonstrated for CCK1R knockout mice (Kopin et al., 1999). In discrete brain areas presence of CCK1R-s has also been demonstrated (Hirosue et al., 1993) although in the brain CCK2R-s represent the dominant and abundant receptor type.

Brain CCK2R-s are generally accepted to have a role in anxiety behavior (Wang et al., 2005), but such receptors in the dorsomedial, paraventricular and ventromedial hypothalamic nuclei might also be mediators of anorexia. Following food intake CCK is released in the hypothalamus (Schick et al., 1990), probably due to signals from stretch of the stomach which signals are conveyed by afferent vagal activity. Exogenous CCK given ICV or to various hypothalamic nuclei suppressed FI in a number of species (Blevins et al., 2000). Similar role for endogenous CCK was demonstrated by postponing satiety via CCK2R antagonist treatment (Dourish et al., 1989). Other studies demonstrated that CCK2R knockout mice are hyperphagic and obese (Clerc et al., 2007) – their hypothalamic NPY expression was also high (Chen et al., 2006). Centrally applied CCK also induced fever-like elevation of body temperature (Szelenyi et al., 1994), and capsaicin desensitization of the abdominal vagus, *i.e.* elimination of CCK-sensing fibers (Petervari et al., 2005) or pretreatment with CCK1R antagonist devazepide (Petervari et al., 2004) prevented the gastric stretch-induced postprandial hypermetabolism and hyperthermia.

We hypothesized that after the young adult age either the peripheral or the central CCK effects may vary with further aging. Age-dependence has already been

demonstrated for the effects of a number of peptides involved in the regulation of food intake, energy balance, thermoregulation and body weight. For example, ICV alpha-MSH has a very strong anorexic and body weight decreasing action in young adult and again in old animals, but not in the middle-aged ones (Petervari et al., 2010). Such alterations in activity may contribute to the explanation of the two basic age-related anomalies of energy balance, *i.e.* the age-related obesity and the late-appearing anorexia of aging that often leads to senile cachexia and sarcopenia – both anomalies having far-reaching health effects. In contrast, for some other peptides, e.g. NPY, ghrelin, orexin (Akimoto and Miyasaka, 2010) and leptin (Scarpace and Tumer, 2001) another pattern of age-related change, a continuous attenuation of the effects has been demonstrated suggesting a stepwise deterioration with age for the regulatory role of the peptide. We assumed that similarly as the role(s) of other peptides, age-related variations of the CCK-dependent regulatory effects possibly contribute to the alterations of energy balance during aging. However, the age-related changes in CCK-efficacy may show either of these patterns. The present data suggest that, depending on the site of action, both patterns are possible for CCK.

Although IP injected CCK suppressed the ingestive behavior in young adult (NF4, NF6) rats, by the age of 12 months this effect of CCK was practically lost. Later on, however, in old animals (NF18, NF24) the anorexic responsiveness to IP administered CCK increased again. The application of one single intraperitoneal dose of CCK (5 μg) in our study (instead of varying the dose in proportion to body weight of the animals) may constitute certain limitations of interpretation of our data. However, when regarding the CCK dose normalized to 100 g body weight, it appears that the highest relative dose in juvenile animals remained inefficient, while the lowest relative doses in old age-groups or middle-aged diet-induced obese rats reduced food intake significantly. In addition, within the young adult group the dose of 1 μg (unpublished data of Balasko et al.) was also able to induce similar and significant suppression of food intake in a similar setting as the 5 μ g dose. Moreover, body weights of all NF adult age-groups were rather similar to one another, while showing significantly different responses to an identical dose of CCK. These latter findings also support our conclusion that CCK-resistance in our middle-aged groups is based on lack of responsiveness and not on an insufficient dose.

Enhanced responsiveness to CCK in the old age-group may be surprising in view of age-related leptin-resistance as there is a well-documented interdependence between effects of these catabolic peptides of mainly peripheral origin (de Lartigue et al., 2012). Although leptin signaling in vagal afferent neurons is required for appropriate satiating effects of CCK, moreover high-fat diet-induced leptin-resistance reduced this satiating effect (at low doses) in young adult rats (de Lartigue et al., 2012), it has not been completely abolished. A higher dose of CCK was shown to inhibit food intake (de Lartigue et al., 2012). As CCK level increases in old age-groups (as discussed later) where some leptin-resistance but not complete abolishment of leptin effects is seen, this higher CCK level may be sufficient to induce anorexia. Melanocortin agonist alpha-MSH (Petervari et al., 2010) that acts downstream of leptin in the hypothalamus shows similar enhancement of anorexigenic efficacy in old age-groups despite leptin-resistance.

The above demonstrated changes in the efficacy to CCK during the course of aging may contribute to insufficient satiety, overeating and obesity in middle-aged rats (age-related obesity) as well as to enhanced satiety and aging anorexia in old animals. As a first approach, the satiety-inducing effect of CCK seems to suggest that it influences the short-term rather than the long-term regulation of food intake. Still, it has been repeatedly reported (Smith et al., 1985) that not so much the number, rather the duration of feeding bouts (determining meal size) is decreased by the peptide what is apparently not fully compensated by feeding frequency. This allows for long-term shifts in energy balance as cumulative effects of changing CCK activity or efficacy. This is likely to be the explanation of obesity in OLETF rats.

It may be of particular importance that – according to most human data – the fasting plasma levels of CCK are higher in the elderly than in young individuals (MacIntosh et al., 2001, Di Francesco et al., 2005, Serra-Prat et al., 2009). This results in suppressed level of hunger that is not altered very much by the relatively small postprandial CCK-release in old persons (Serra-Prat et al., 2009). A period of caloric restriction is poorly compensated in elderly men (Winkels et al., 2011). Animal experiments similarly show higher CCK levels in old animals: in synaptosomes of brain samples from old rats the CCK-content was higher than in young ones, although the CCK-release in brain samples upon stimulation was smaller (Ohta et al., 1995).

There are limited and controversial data concerning CCK production/effect (and effects of other neuropeptides) in high-fat diet induced obese rat models even in the

young adult age-group. Such dietary interventions were shown to lead to elevation of plasma CCK-concentration in rats (Li et al., 2011). Nevertheless, various effects of exogenous CCK are not necessarily simultaneously enhanced (Little et al., 2008, How et al., 2011). Other reports described suppression of gastrointestinal CCK gene- and protein expression (as well as those of peptide YY and glucagon-like peptide-1) (Duca et al., 2013) and reduced satiety in response to CCK and bombesine (Covasa and Ritter, 1998, Torregrossa and Smith, 2003). No relevant information regarding age-related alterations in CCK level or activity are available in high-fat diet-induced obese rodent models.

The effects of long-term caloric restriction have not been investigated on peripheral CCK expression or activity either in young adult rats or during the course of aging. According to our previous observations caloric restriction appears to enhance some aspects of neuropeptide effects (Soos et al., 2010, Soos et al., 2011).

In the present studies CCK-responses were decreased in dietary obese rats already at the age of 6 months (HF6), unlike the pronounced anorexic CCK effects in normally fed rats of the same age (NF6). Contrary to this, in CR rats of probably low plasma CCK levels CCK-resistance did not develop even at the age of 12 months (CR12), when normally fed middle-aged (NF12) rats were "resistant" to CCK-anorexia. In NF rats a rebound of CCK-responsiveness was observed with aging after middle-age (*i.e.* in NF18-NF24 groups), in HF rats the rebound was present already at the age of 12 months. Apparently, caloric restriction seemed to postpone, obesity to speed up the age-related changes in CCK-responsiveness.

It may be concluded that peripheral CCK-actions seem to be important in the overall energy balance by determining food intake and consequently the nutritional state. These actions change with phases of aging and they also depend on body composition.

The ICV injected CCK suppressed the ingestive behavior in young adult rats, but – unlike in case of IP administration – this effect of the peptide became gradually weaker with the aging process and by the age of 24 months (NF24) there was practically no effect. Apparently, not only the anorexic, but also the hypermetabolic and hyperthermic effects of ICV CCK vanished with increasing age. This pattern of change in neuropeptide effects is characteristic for some peptides like NPY, ghrelin, orexin, etc. (Akimoto and Miyasaka, 2010). Considering that a decrease in metabolic rate is characteristic for old age (McGandy et al., 1966), the lack of effect of centrally applied

CCK suggests that the central CCK activity may have but little importance in determining metabolic rate, at least in old animals, while the lack of anorexic effect in old rats suggests that the age-related anorexia is probably also independent of central CCK activity.

Altogether, cerebral CCK2R-s are likely to have some catabolic role in energy balance of young adult animals: the CCK2R-dependent postprandial anorexia and hypermetabolism possibly play a role in the metabolic adaptation to calorie intake, to maintain energy equilibrium. Studies on the control of food intake in older men have shown that, unlike in their young counterparts, an excessive calorie containing diet of the same length was not readily compensated following the dietary period – this is part of the phenomenon known as "dysorexia" of the elderly (Roberts et al., 1994).

Central CCK (CCK2R-s) may also participate in fever and sickness behavior (Szelenyi et al., 1994, Weiland et al., 2007). Aging is associated with diminished fever response (Buchanan et al., 2003).

Central CCK-actions seem to be important in the overall energy balance by determining fever-like metabolic response with additional anorexic effect. According to our data these effects decline with aging. Our present findings raise the hypothesis that age-related decline in the central hyperthermic and anorexic effects of CCK may contribute to the age-related diminishment of fever, alterations in sickness behavior and insufficiency of metabolic adaptation to feeding.

Although CCK of peripheral origin also acts in the brain, on its CCK1R-s at various nuclei, the points of action of this CCK and those of centrally applied/released CCK (acting on CCK2R-s of the brain) cannot be identical as shown by the opposite metabolic/thermal effects and by the fact that the age-related changes in their efficacy are different.

6.2 Alarin

The first part of the experiments investigated the thermoregulatory effects of alarin, a 25 amino-acid peptide, the newest member of the orexigenic galanin peptide family that shows structural and functional similarities to 60-amino-acid GALP (Eberhard et al., 2012, Fraley et al., 2012, Webling et al., 2012, Fraley et al., 2013). Alarin immunoreactivity shows a broader expression pattern in the murine brain than that of GALP, including different nuclei of the hypothalamus (DMH, lateral hypothalamus, PVN) (Eberhard et al., 2012). Kofler and coworkers recently reported

that an ICV injection of alarin significantly increased the expression of the immediate early gene *c-fos*, a marker for neuronal activation in different brain regions including the PVN, DMN and the arcuate nucleus of male rats (Van Der Kolk et al., 2010). Alarin also induces NPY release from hypothalamic explants similar to GALP, suggesting that alarin may exert its effect on feeding mediated by similar pathways (Boughton et al., 2010). However, no conclusive evidence (such as successful application of NPY antagonists to inhibit alarin effects) have been proposed for NPY mediation of alarin actions**.**

As alarin (unlike GALP) has no detectable affinity towards any of the known three galanin receptor subtypes (Santic et al., 2007, Boughton et al., 2010), its actions are assumed to be mediated by a separate or an as yet unidentified receptor.

Although a previous report failed to reveal any change in body temperature upon an ICV alarin injection in freely moving mice using biotelemetry (Fraley et al., 2012), and no change in oxygen consumption has been detected in adult male freely moving Long-Evans rats upon a similar alarin administration either (in freely moving rats locomotion itself may raise metabolic rate (Van Der Kolk et al., 2010), as yet no conclusive thermoregulatory tests involving alarin have been conducted in rats.

The present data show that centrally administered alarin appears to elicit a slow but significant hypermetabolic, hyperthermic thermoregulatory response, further enhanced by a suppression of heat loss in rats. The rate of this thermoregulatory response is similar at different doses and at a wide range of ambient temperatures (from 15 to 28 °C). Similarly to food intake-related observations (Fraley et al., 2013), the thermoregulatory actions of alarin were also lost when the first 5 amino acids were removed, and the truncated peptide also acted as an antagonist in our thermoregulatory tests.

Based on the long latency of the central hyperthermic response and on previous reports about cutaneous vasoconstriction induced by peripheral alarin injections (Santic et al., 2007), the question arises whether the observed delayed thermoregulatory effects of full-length alarin are partly due to its peripheral actions following passage of the peptide from the lateral ventricle to the peripheral circulation. However, our data show that direct peripheral IP administration of the peptide at doses used also ICV failed to show any thermoregulatory response.

With regard to the potential NPY mediation of alarin effects, the thermoregulatory effects of alarin appear to differ from those of NPY in a cool environment, where acute

ICV NPY administration elicits acute hypometabolism and hypothermia before leading to some delayed rise in body temperature. Moreover, NPY does not induce vasoconstriction within a similar range of ambient temperatures (Szekely and Szelenyi, 2005).

The thermoregulatory effects of alarin described in our study appear to be somewhat similar to those of GALP. Upon acute ICV GALP injection Tc rises promptly lasting for 6-8 hours in rats (Lawrence et al., 2002). So far, GALP seems to elicit a fever-like hyperthermia (Szekely and Szelenyi, 1979), because it elicits hypermetabolism associated with a reduced heat loss (Kageyama et al., 2013). Lawrence and her coworkers found that IP applied flurbiprofen, a non-selective COX inhibitor, reduced the increase in core body temperature after ICV GALP injection (Lawrence et al., 2002) indicating the involvement of prostaglandins. Fever involves activation of the arachidonic acid cascade and finally synthesis of prostaglandin E_2. Therefore, fever can be suppressed by selective or non-selective COX inhibitor substances (Harvey and Milton, 1975). Moreover, similarly to endotoxin fever, GALP-induced hyperthermia also appears to be mediated by interleukin-1 (Lawrence et al., 2002, Man and Lawrence, 2008b). Similarities between the thermoregulatory effects of alarin and GALP and also the delay in the onset of alarin-hyperthermia raised the potential involvement of prostaglandins as secondary mediators in the responses elicited by alarin.

In our study, both the non-selective COX inhibitor indomethacin and the relatively selective COX-2 inhibitor meloxicam reduced hypermetabolic and hyperthermic effects of alarin, suggesting potential involvement of the peptide in fever. Moreover, fever appears as a component of sickness behavior accompanied also by anorexia (Johnson, 2002). In addition, the fever-like response to alarin would characterize a catabolic (i.e. hypermetabolic and anorexigenic) rather than an anabolic mediator.

Indeed, regarding FI-related effects, in our present study alarin failed to induce FI in the daytime period, but it also led to a reduction of cumulative 24-h FI. Surprisingly, alarin showed its anorexigenic character also in other experimental settings. The ICV injection of the peptide given at the onset of the active nighttime period slowly but strongly suppressed spontaneous nighttime cumulative FI without any compensation in the following daytime period. After 24-h fasting, ICV alarin injection significantly decreased re-feeding FI and this anorexigenic effect persisted up to 24 hours. These slow anorexigenic responses resemble the dynamics of the thermoregulatory changes

induced by alarin. Two earlier studies showed some orexigenic action of alarin in rats. One of them was performed in younger (6 weeks old) Long Evans rats (Van Der Kolk et al., 2010). Although younger juvenile age groups of rats may exhibit different reactions compared to those of young adult (3 months old) animals (Petervari et al., 2010), in our present study similar doses of alarin failed to induce any FI even in 6 weeks old Wistar rats or in similar juvenile Long Evans rats. The difference between the earlier and present observations concerning FI-related effects of alarin may be explained by the different methods of measurements. Spontaneous daytime 180-min FI measured by our specialized FeedScale system allowing precise automated measurement of consumed powdered rat chow without spillage remained below 1 g, whereas the earlier study (Van Der Kolk et al., 2010) detected a much higher value (about 3 g at 180 min) under similar conditions. In addition, detected FI difference between control and alarin-treated groups remained in the region of 1-2 g. The results of the other study obtained from the experiments in adult male Wistar rats demonstrated orexigenic action of alarin only during the first hour upon injection of a very high dose (30 nmol or 84.6 μg) of the peptide into the third cerebral ventricle. There was no significant effect of alarin on FI at any other dose or time-point studied (Boughton et al., 2010).

In summary, according to our present observations, alarin seems to be a central catabolic peptide. Based on its combined parallel anorexigenic and fever-like, prostaglandin-mediated hyperthermic/hypermetabolic effects the potential involvement of alarin in sickness behavior may be assumed.

7. Perspectives

7.1 CCK

Our results raise the hypothesis that peripheral and central receptor mechanisms of CCK play distinctly differential roles in the regulation of energy balance. Short-term regulation of FI and the characteristic shifts in the long-term regulation of energy balance in the course of life (depending on the nutritional state) appear to be connected mainly with peripheral receptors, while the activity of central receptors may present a metabolic compensation for calorie intake and defense against energy accumulation in young age-groups. In old rats the loss of metabolic responsiveness to centrally applied CCK appears to be reasonable, since these animals tend to lose weight anyhow, and a high metabolic response to calorie intake would speed up this unfavorable process (this

is exactly what may be connected with the increased sensitivity to peripheral CCK). It may be of interest to see whether the loss of metabolic responsiveness is similar in obese old rats with abundant energy reserves some of which may be lost without severe consequences. Specific antagonists of peripheral and central CCK receptors would be useful in the analysis of differential CCK functions during the course of aging.

7.2 Alarin

In our experiments we demonstrated the catabolic character of alarin in young adult Wistar rat model and we performed the experiments also in young Long Evans rats. In the future, changes in effect of alarin during aging will be examined. It would be also interesting, if these effects may vary in animals with different nutritional states.

A new perspective could be the intranasal administration of alarin to Wistar rats, as it has been seen in case of GALP. It could be a new administration method for alarin as antiobesity drug, but further some features of this peptide need to clarify.

8. Summary

Both the peripheral and the central CCK-effects (anorexigenic as well as hypermetabolic effects) are age-dependent. The peripheral effects change with age and may contribute to the age-related phasic changes in overall energy balance and consequent changes in body weight, i.e., to the age-related obesity in middle-aged and the aging anorexia in old subjects. The central effects may change in a way that the metabolic compensation of calorie intake (postprandial hypermetabolism) becomes attenuated or is lost completely in old age. Diet-induced obesity appears to accelerate, caloric restriction to slow down these age-related processes.

- Accordingly, our main conclusions concerning effects of CCK on the regulation energy homeostasis:
- Both peripheral and central CCK-effects are age-dependent.
- Peripheral anorexigenic CCK effects are low in middle-aged, but they are enhanced in old rats.
- Peripheral CCK plays a role in the development of midle-aged obesity and aging anorexia.
- Central CCK plays a role in postprandial hypermetabolism.
- Central hyperthermic and anorexigenic CCK effects decline with age.

1. These new findings are published in: Marta Balasko, Ildiko Rostas, Nora Furedi, **Alexandra Miko**, Peter Cseplo, Margit Koncsecsko-Gaspar, Szilvia Soos, Miklos Szekely, Erika Petervari: Age and nutritional state influence the effects of cholecystokinin on energy balance, Experimental Gerontology, 48: 11, 2013, 1180–1188 IF: 3.529

Concerning effects of alarin on the regulation of energy homeostasis, our main conclusions are the followings:

- Alarin elicits a centrally coordinated, fever-like hyperthermic response in rats.
- Inhibition of prostaglandin synthesis suppresses thermoregulatory effects of alarin.
- Alarin reduces spontaneous night-time and fasting-induced re-feeding food intake.
- Alarin appears to be a catabolic neuropeptide.

- Alarin may participate in the development of sickness behavior.

2. These new findings are published in: **Alexandra Miko**, Peter Balla, Judit Tenk, Marta Balasko, Szilvia Soos, Miklos Szekely, Susanna Brunner, Barbara Kofler, Erika Petervari: Thermoregulatory effect of alarin, a new member of the galanin peptide family, 2014, Temperature 1:1, 1–6.

   Alexandra Miko, Nora Furedi, Judit Tenk, Ildiko Rostas, Szilvia Soos, Margit Solymar, Miklos Szekely, Marta Balasko, Susanne Brunner, Barbara Kofler, Erika Petervari: Acute central effects of alarin on the regulation on energy homeostasis, Neuropeptides. 2016, S0143-4179(16)30087-7. IF: 2.726

9. Acknowledgements

All the work presented in this thesis could not have been carried out without the enormous help from numerous people, to whom I owe a great debt of gratitude. First of all I would like to thank my supervisor Dr. Erika Petervari for her continuous support, guidance and irreplaceable help in summarizing my thesis

Special thanks must be paid to the members of the research group, Dr. Marta Balasko, prof. Miklos Szekely and Nora Furedi. I benefited greatly from invaluable discussions with them. I would like to thank the assistants of the Research Group for the Regulation Energy Homeostasis and Experimental Gerontology of the Institute for Translational Medicine, to Andrea Jech-Mihalffy, Adrienn Boka-Kissne and Margit Koncsecsko-Gaspar for teaching me the special experimental methods and for working together as a team.

I would like to thank Prof. Barbara Kofler (Laura Bassi Centre of Expertise – THERAPEP, Department of Pediatrics, Paracelsus Medical University, Salzburg; Austria) for her generous help, as my work on alarin was inspired by her groundbreaking research regarding this newly discovered peptide. I am also grateful for the gift of alarin and related peptides for my experiments.

Special thanks must be paid to my friends, to my parents, my sisters, my boyfriend and other members of my family for their love and for supporting and encouraging me through all these years.

10. Abbreviations

ARC – nucleus arcuatus
alpha-MSH – alpha-melanocyte stimulating hormone
BW – body weight
CCK – cholecystokinin
CCK1R – cholecystokinin-1 receptor
CCK2R – cholecystokinin-2 receptor
COX – cyclooxygenase
CR – caloric-restricted
DMH – dorsomedial hypothalamic nucleus
FI – food intake
GALP – galanin-like peptide
Gal-R – galanin receptor
HF – high fat
HL – heat loss
HLI – heat loss index
ICV – intracerebroventricular
IP – intraperitoneal
LH – luteinizing hormone
MR – metabolic rate
NF – normally fed
NPY – neuropeptide Y
NS – non-significant
NTS – nucleus tractus solitarii
PGE1 – prostaglandin E1
PVN – nucleus paraventricularis
RQ – respiratory quotient
STPD – standard temperature and pressure
Ta – ambient temperature
Tc – core body temperature
Ts – tail skin temperature
VMH – ventromedial hypothalamus
VO_2 – oxygen consumption

11. References

Akimoto S, Miyasaka K (2010) Age-associated changes of appetite-regulating peptides. Geriatrics & Gerontology International 10 Supplementum 1:S107-119.

Balasko M, Rostas I, Tenk J, Soos S, Petervari E (2016) Leptin and aging in animal models. In: Handbook of Models for Human Aging 2nd Edition, Edited by:P. Michael Conn, Elsevier, in press.

Balasko M, Soos S, Parniczky A, Koncsecsko-Gaspar M, Szekely M, Petervari E (2012) Anorexic effect of peripheral cholecystokinin (CCK) varies with age and body composition (short communication). Acta physiologica Hungarica 99:166-172.

Beck B, Stricker-Krongrad A, Nicolas JP, Burlet C (1992) Chronic and continuous intracerebroventricular infusion of neuropeptide Y in Long-Evans rats mimics the feeding behaviour of obese Zucker rats. International Journal of Obesity and Related Metabolic Disorders : Journal of the International Association for the Study of Obesity 16:295-302.

Berthoud H-R (2004) The caudal brainstem and the control of food intake and energy balance, E.M. Stricker, S.C. Woods (Eds.), Handbook of Behavioral Neurobiology, Plenum, New York, 195-240.

Bi S, Chen J, Behles RR, Hyun J, Kopin AS, Moran TH (2007) Differential body weight and feeding responses to high-fat diets in rats and mice lacking cholecystokinin 1 receptors. American Journal of Physiology Regulatory, Integrative and Comparative Physiology 293:R55-63.

Blessing WW (1997) The Lower Brainstem and Bodily Homeostasis, (Ch. 7, Eating and metabolism) Oxford University,Press, New York, 323-372.

Blevins JE, Stanley BG, Reidelberger RD (2000) Brain regions where cholecystokinin suppresses feeding in rats. Brain Research 860:1-10.

Boughton CK, Murphy KG (2013) Can neuropeptides treat obesity? A review of neuropeptides and their potential role in the treatment of obesity. British Journal of Pharmacology 170:1333-1348.

Boughton CK, Patterson M, Bewick GA, Tadross JA, Gardiner JV, Beale KE, Chaudery F, Hunter G, Busbridge M, Leavy EM, Ghatei MA, Bloom SR, Murphy KG (2010) Alarin stimulates food intake and gonadotrophin release in male rats. British Journal of Pharmacology 161:601-613.

Buchanan JB, Peloso E, Satinoff E (2003) Thermoregulatory and metabolic changes during fever in young and old rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 285:R1165-1169.

Calam J, Ellis A, Dockray GJ (1982) Identification and measurement of molecular variants of cholecystokinin in duodenal mucosa and plasma. Diminished concentrations in patients with celiac disease. The Journal of Clinical Investigation 69:218-225.

Cano V, Merino B, Ezquerra L, Somoza B, Ruiz-Gayo M (2008) A cholecystokinin-1 receptor agonist (CCK-8) mediates increased permeability of brain barriers to leptin. British Journal of Pharmacology 154:1009-1015.

Chapman IM, MacIntosh CG, Morley JE, Horowitz M (2002) The anorexia of ageing. Biogerontology 3:67-71.

Chen H, Kent S, Morris MJ (2006) Is the CCK2 receptor essential for normal regulation of body weight and adiposity? The European Journal of Neuroscience 24:1427-1433.

Cheng CA, Geoghegan JG, Lawson DC, Berlangieri SU, Akwari O, Pappas TN (1993) Central and peripheral effects of CCK receptor antagonists on satiety in dogs. The American Journal of Physiology 265:G219-223.

Clerc P, Coll Constans MG, Lulka H, Broussaud S, Guigne C, Leung-Theung-Long S, Perrin C, Knauf C, Carpene C, Penicaud L, Seva C, Burcelin R, Valet P, Fourmy D, Dufresne M (2007) Involvement of cholecystokinin 2 receptor in food intake regulation: hyperphagia and increased fat deposition in cholecystokinin 2 receptor-deficient mice. Endocrinology 148:1039-1049.

Corp ES, McQuade J, Moran TH, Smith GP (1993) Characterization of type A and type B CCK receptor binding sites in rat vagus nerve. Brain research 623:161-166.

Covasa M, Ritter RC (1998) Rats maintained on high-fat diets exhibit reduced satiety in response to CCK and bombesin. Peptides 19:1407-1415.

Crawley JN (1999) The role of galanin in feeding behavior. Neuropeptides 33:369-375.

de Lartigue G, Barbier de la Serre C, Espero E, Lee J, Raybould HE (2012) Leptin resistance in vagal afferent neurons inhibits cholecystokinin signaling and satiation in diet induced obese rats. PloS One 7:e32967.

de Lartigue G, Dimaline R, Varro A, Dockray GJ (2007) Cocaine- and amphetamine-regulated transcript: stimulation of expression in rat vagal afferent neurons by

cholecystokinin and suppression by ghrelin. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 27:2876-2882.

Della-Fera MA, Baile CA (1979) Cholecystokinin octapeptide: continuous picomole injections into the cerebral ventricles of sheep suppress feeding. Science 206:471-473.

Deschenes RJ, Lorenz LJ, Haun RS, Roos BA, Collier KJ, Dixon JE (1984) Cloning and sequence analysis of a cDNA encoding rat preprocholecystokinin. Proceedings of the National Academy of Sciences of the United States of America 81:726-730.

Deschodt-Lanckman M, Bui ND, Noyer M, Christophe J (1981) Degradation of cholecystokinin-like peptides by a crude rat brain synaptosomal fraction: a study by high pressure liquid chromatography. Regulatory Peptides 2:15-30.

Di Francesco V, Zamboni M, Dioli A, Zoico E, Mazzali G, Omizzolo F, Bissoli L, Solerte SB, Benini L, Bosello O (2005) Delayed postprandial gastric emptying and impaired gallbladder contraction together with elevated cholecystokinin and peptide YY serum levels sustain satiety and inhibit hunger in healthy elderly persons. The Journal of Gerontology, Series A, Biology Sciences and Medical Sciences 60:1581-1585.

Dockray GJ (2009) Cholecystokinin and gut-brain signalling. Regulatory peptides 155:6-10.

Donovan MJ, Paulino G, Raybould HE (2007) CCK(1) receptor is essential for normal meal patterning in mice fed high fat diet. Physiology & Behavior 92:969-974.

Dourish CT, Rycroft W, Iversen SD (1989) Postponement of satiety by blockade of brain cholecystokinin (CCK-B) receptors. Science 245:1509-1511.

Duca FA, Swartz TD, Sakar Y, Covasa M (2013) Decreased intestinal nutrient response in diet-induced obese rats: role of gut peptides and nutrient receptors. International Journal of Obesity 37:375-381.

Eberhard N, Mayer C, Santic R, Navio RP, Wagner A, Bauer HC, Sperk G, Boehm U, Kofler B (2012) Distribution of alarin immunoreactivity in the mouse brain. Journal of Molecular Neuroscience 46:18-32.

Eberhard N, Schmidhuber S, Rauch MI, Sperk G, Kofler B (2007) Immunohistochemical distribution of the novel peptide alarin in the adult rat brain. FEBS Journal 274, 204.

Eberlein GA, Eysselein VE, Goebell H (1988) Cholecystokinin-58 is the major molecular form in man, dog and cat but not in pig, beef and rat intestine. Peptides 9:993-998.

Edwards GL, Ladenheim EE, Ritter RC (1986) Dorsomedial hindbrain participation in cholecystokinin-induced satiety. American Journal of Physiology 251:R971-977.

Evans H, Baumgartner M, Shine J, Herzog H (1993) Genomic organization and localization of the gene encoding human preprogalanin. Genomics 18:473-477.

Fraley GS, Leathley E, Lundy N, Chheng E, King I, Kofler B (2012) Effects of alarin on food intake, body weight and luteinizing hormone secretion in male mice. Neuropeptides 46:99-104.

Fraley GS, Leathley E, Nickols A, Gerometta E, Coombs E, Colton S, Gallemore S, Lindberg A, Kofler B (2013) Alarin 6-25Cys antagonizes alarin-specific effects on food intake and luteinizing hormone secretion. Neuropeptides 47:37-41.

Gabriely I, Ma XH, Yang XM, Atzmon G, Rajala MW, Berg AH, Scherer P, Rossetti L, Barzilai N (2002) Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? Diabetes 51:2951-2958.

Gaudreau P, Quirion R, St-Pierre S, Pert CB (1983) Characterization and visualization of cholecystokinin receptors in rat brain using [3H]pentagastrin. Peptides 4:755-762.

Gibbs J, Young RC, Smith GP (1973) Cholecystokinin decreases food intake in rats. Journal of Comparative and Physiological Psychology 84:488-495.

Grill HJ, Smith GP (1988) Cholecystokinin decreases sucrose intake in chronic decerebrate rats. The American Journal of Physiology 254:R853-856.

Gundlach AL, Burazin TC, Larm JA (2001) Distribution, regulation and role of hypothalamic galanin systems: renewed interest in a pleiotropic peptide family. Clinical and Experimental Pharmacology & Physiology 28:100-105.

Harper AA, Raper HS (1943) Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. The Journal of Physiology 102:115-125.

Hill DR, Campbell NJ, Shaw TM, Woodruff GN (1987) Autoradiographic localization and biochemical characterization of peripheral type CCK receptors in rat CNS using highly selective nonpeptide CCK antagonists. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 7:2967-2976.

Hill DR, Shaw TM, Graham W, Woodruff GN (1990a) Autoradiographical detection of cholecystokinin-A receptors in primate brain using 125I-Bolton Hunter CCK-8 and 3H-MK-329. The Journal of Neuroscience: The Official Journal of the Society for Neuroscience 10:1070-1081.

Hill DR, Singh L, Boden P, Pinnock GN, Hughes J (1992) Detection of CCK receptor subtypes in mammalian brain using highly selective non-peptide antagonists. In Multiple cholecystokinin receptors in the CNS, by Dourish C.T., Cooper S.J., Iversen S.D., Iversen L.L., Oxford: Oxford University Press S.57-76.

Hill DR, Woodruff GN (1990b) Differentiation of central cholecystokinin receptor binding sites using the non-peptide antagonists MK-329 and L-365,260. Brain Research 526:276-283.

Hirosue Y, Inui A, Teranishi A, Miura M, Nakajima M, Okita M, Nakajima Y, Himori N, Baba S, Kasuga M (1993) Cholecystokinin octapeptide analogues suppress food intake via central CCK-A receptors in mice. The American Journal of Physiology 265:R481-486.

Hokfelt T, Aman K, Arvidsson U, Bedecs K, Ceccatelli S, Hulting AL, Langel U, Meister B, Pieribone V, Bartfai T (1992) Galanin message-associated peptide (GMAP)- and galanin-like immunoreactivities: overlapping and differential distributions in the rat. Neuroscience Letters 142:139-142.

Hokfelt T, Tatemoto K (2010) Galanin: a multitalented neuropeptide. Exs 102:1-5.

Holub BS, Rauch I, Radner S, Sperl W, Hell M, Kofler B (2011) Effects of galanin message-associated peptide and neuropeptide Y against various non-albicans Candida strains. International Journal of Antimicrobial Agents 38:76-80.

How JM, Fam BC, Verberne AJ, Sartor DM (2011) High-fat diet is associated with blunted splanchnic sympathoinhibitory responses to gastric leptin and cholecystokinin: implications for circulatory control. American Journal of Physiology Heart and Circulatory Physiology 300:H961-967.

Ivy A.C. OE (1928a) Observation on the cause of gall-bladder contraction and evacuation. Proceedings of the Society for Experimental Biology and Medicine 251-252.

Ivy A.C. OE (1928b) Contraction and evacuation of gall-bladder caused by highly purified "sekretin" preparation. American Medical Association 90 445-446.

Johnson RW (2002) The concept of sickness behavior: a brief chronological account of four key discoveries. Veterinary Immunology and Immunopathology 87:443-450.

Jorpes E, Mutt V (1966) Cholecystokinin and pancreozymin, one single hormone? Acta Physiologica Scandinavica 66:196-202.

Kageyama H, Endo K, Osaka T, Watanabe J, Wang LH, Ito K, Suzuki M, Sakagami J, Takenoya F, Shioda S (2013) Galanin-like peptide (GALP) facilitates thermogenesis via synthesis of prostaglandin E2 by astrocytes in the periventricular zone of the third ventricle. Journal of Molecular Neuroscience : MN 50:443-452.

Kageyama H, Shiba K, Hirako S, Wada N, Yamanaka S, Nogi Y, Takenoya F, Nonaka N, Hirano T, Inoue S, Shioda S (2016) Anti-obesity effect of intranasal administration of galanin-like peptide (GALP) in obese mice. Scientific Reports 6:28200.

Kapas L, Obal F, Jr., Penke B, Obal F (1987) Cholecystokinin-octapeptide-induced hypothermia in rats: dose-effect and structure-effect relationships, effect of ambient temperature, pharmacological interactions and tolerance. Neuropharmacology 26:131-137.

Kaplan LM, Spindel ER, Isselbacher KJ, Chin WW (1988) Tissue-specific expression of the rat galanin gene. Proceedings of the National Academy of Sciences of the United States of America 85:1065-1069.

Kauffman AS, Buenzle J, Fraley GS, Rissman EF (2005) Effects of galanin-like peptide (GALP) on locomotion, reproduction, and body weight in female and male mice. Hormones and Behavior 48:141-151.

Kmiec Z, Petervari E, Balasko M, Szekely M (2013) Anorexia of aging. Vitamins and Hormones 92:319-355.

Knorr C, Marks D, Gerstberger R, Muhlradt PF, Roth J, Rummel C (2010) Peripheral and central cyclooxygenase (COX) products may contribute to the manifestation of brain-controlled sickness responses during localized inflammation induced by macrophage-activating lipopeptide-2 (MALP-2). Neuroscience Letters 479:107-111.

Kopelman PG (2000) Obesity as a medical problem. Nature 404:635-643.

Kopin AS, Mathes WF, McBride EW, Nguyen M, Al-Haider W, Schmitz F, Bonner-Weir S, Kanarek R, Beinborn M (1999) The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. The Journal of Clinical Investigation 103:383-391.

Krasnow SM, Fraley GS, Schuh SM, Baumgartner JW, Clifton DK, Steiner RA (2003) A role for galanin-like peptide in the integration of feeding, body weight regulation, and reproduction in the mouse. Endocrinology 144:813-822.

Kuramochi M, Onaka T, Kohno D, Kato S, Yada T (2006) Galanin-like peptide stimulates food intake via activation of neuropeptide Y neurons in the hypothalamic dorsomedial nucleus of the rat. Endocrinology 147:1744-1752.

Kyrkouli SE, Stanley BG, Leibowitz SF (1986) Galanin: stimulation of feeding induced by medial hypothalamic injection of this novel peptide. European Journal of Pharmacology 122:159-160.

Lacourse KA, Swanberg LJ, Gillespie PJ, Rehfeld JF, Saunders TL, Samuelson LC (1999) Pancreatic function in CCK-deficient mice: adaptation to dietary protein does not require CCK. The American Journal of Physiology 276:G1302-1309.

Lang R, Berger A, Santic R, Geisberger R, Hermann A, Herzog H, Kofler B (2005) Pharmacological and functional characterization of galanin-like peptide fragments as potent galanin receptor agonists. Neuropeptides 39:179-184.

Larm JA, Gundlach AL (2000) Galanin-like peptide (GALP) mRNA expression is restricted to arcuate nucleus of hypothalamus in adult male rat brain. Neuroendocrinology 72:67-71.

Lawrence CB, Baudoin FM, Luckman SM (2002) Centrally administered galanin-like peptide modifies food intake in the rat: a comparison with galanin. Journal of Neuroendocrinology 14:853-860.

Leibowitz SF (1998) Differential functions of hypothalamic galanin cell grows in the regulation of eating and body weight. Annals of the New York Academy of Sciences 863:206-220.

Leibowitz SF (2005) Regulation and effects of hypothalamic galanin: relation to dietary fat, alcohol ingestion, circulating lipids and energy homeostasis. Neuropeptides 39:327-332.

Lewis MJ, Johnson DF, Waldman D, Leibowitz SF, Hoebel BG (2004) Galanin microinjection in the third ventricle increases voluntary ethanol intake. Alcoholism, Clinical and Experimental Research 28:1822-1828.

Li J, Ma W, Wang S (2011) Slower gastric emptying in high-fat diet induced obese rats is associated with attenuated plasma ghrelin and elevated plasma leptin and cholecystokinin concentrations. Regulatory Peptides 171:53-57.

Little TJ, Feltrin KL, Horowitz M, Meyer JH, Wishart J, Chapman IM, Feinle-Bisset C (2008) A high-fat diet raises fasting plasma CCK but does not affect upper gut motility, PYY, and ghrelin, or energy intake during CCK-8 infusion in lean men. American Journal of Physiology; Regulatory, Integrative and Comparative Physiology 294:R45-51.

Lo CM, King A, Samuelson LC, Kindel TL, Rider T, Jandacek RJ, Raybould HE, Woods SC, Tso P (2010) Cholecystokinin knockout mice are resistant to high-fat diet-induced obesity. Gastroenterology 138:1997-2005.

MacIntosh CG, Andrews JM, Jones KL, Wishart JM, Morris HA, Jansen JB, Morley JE, Horowitz M, Chapman IM (1999) Effects of age on concentrations of plasma cholecystokinin, glucagon-like peptide 1, and peptide YY and their relation to appetite and pyloric motility. The American Journal of Clinical Nutrition 69:999-1006.

MacIntosh CG, Morley JE, Wishart J, Morris H, Jansen JB, Horowitz M, Chapman IM (2001) Effect of exogenous cholecystokinin (CCK)-8 on food intake and plasma CCK, leptin, and insulin concentrations in older and young adults: evidence for increased CCK activity as a cause of the anorexia of aging. Journal of Clinical Endocrinology and Metabolism 86:5830-5837.

Man PS, Lawrence CB (2008a) The effects of galanin-like peptide on energy balance, body temperature and brain activity in the mouse and rat are independent of the GALR2/3 receptor. Journal of Neuroendocrinology 20:128-137.

Man PS, Lawrence CB (2008b) Interleukin-1 mediates the anorexic and febrile actions of galanin-like Peptide. Endocrinology 149:5791-5802.

Martin SM, Wilson BC, Chen X, Takahashi Y, Poulin P, Pittman QJ (2000) Vagal CCK and 5-HT(3) receptors are unlikely to mediate LPS or IL-1beta-induced fever. American Journal of Physiology; Regulatory, Integrative and Comparative Physiology 279:R960-965.

McGandy RB, Barrows CH, Jr., Spanias A, Meredith A, Stone JL, Norris AH (1966) Nutrient intakes and energy expenditure in men of different ages. Journal of Gerontology 21:581-587.

Mennicken F, Hoffert C, Pelletier M, Ahmad S, O'Donnell D (2002) Restricted distribution of galanin receptor 3 (GalR3) mRNA in the adult rat central nervous system. Journal of Chemical Neuroanatomy 24:257-268.

Mercer JG, Lawrence CB (1992) Selectivity of cholecystokinin (CCK) receptor antagonists, MK-329 and L-365,260, for axonally-transported CCK binding sites on the rat vagus nerve. Neuroscience Letters 137:229-231.

Mercer LD, Le VQ, Nunan J, Jones NM, Beart PM (2000) Direct visualization of cholecystokinin subtype2 receptors in rat central nervous system using anti-peptide antibodies. Neuroscience Letters 293:167-170.

Mitchell V, Bouret S, Howard AD, Beauvillain JC (1999) Expression of the galanin receptor subtype Gal-R2 mRNA in the rat hypothalamus. Journal of Chemical Neuroanatomy 16:265-277.

Mitchell V, Habert-Ortoli E, Epelbaum J, Aubert JP, Beauvillain JC (1997) Semiquantitative distribution of galanin-receptor (GAL-R1) mRNA-containing cells in the male rat hypothalamus. Neuroendocrinology 66:160-172.

Mitsukawa K, Lu X, Bartfai T (2008) Galanin, galanin receptors and drug targets. Cellular and Molecular Life Sciences 65:1796-1805.

Miyasaka K, Ichikawa M, Ohta M, Kanai S, Yoshida Y, Masuda M, Nagata A, Matsui T, Noda T, Takiguchi S, Takata Y, Kawanami T, Funakoshi A (2002) Energy metabolism and turnover are increased in mice lacking the cholecystokinin-B receptor. Journal of Nutrition 132:739-741.

Mohn C, Lomniczi A, Faletti A, Scorticati C, Elverdin JC, McCann SM, Rettori V (2001) Effects of aminoguanidine and meloxicam on nitric oxide and prostaglandin E production induced by lipopolysaccharide in the hypothalamus and anterior pituitary of the rat. Neuroimmunomodulation 9:276-285.

Moran TH, Chen J, Bi S (2006) Cholecystokinin and satiety, A.J. Kastin (Ed). Handbook of Biologically Active Peptides, Elsevier/Academic Press, Amsterdam, 961-968.

Moran TH, Kinzig KP (2004) Gastrointestinal satiety signals II. Cholecystokinin. American Journal of Physiology; Gastrointestinal and Liver Physiology 286:G183-188.

Moran TH, Robinson PH, Goldrich MS, McHugh PR (1986) Two brain cholecystokinin receptors: implications for behavioral actions. Brain Research 362:175-179.

Morisset J, Levenez F, Corring T, Benrezzak O, Pelletier G, Calvo E (1996) Pig pancreatic acinar cells possess predominantly the CCK-B receptor subtype. The American Journal of Physiology 271:E397-402.

Ohta M, Tanaka Y, Masuda M, Miyasaka K, Funakoshi A (1995) Impaired release of cholecystokinin (CCK) from synaptosomes in old rats. Neuroscience Letters 198:161-164.

Ohtaki T, Kumano S, Ishibashi Y, Ogi K, Matsui H, Harada M, Kitada C, Kurokawa T, Onda H, Fujino M (1999) Isolation and cDNA cloning of a novel galanin-like peptide (GALP) from porcine hypothalamus. The Journal of Biological Chemistry 274:37041-37045.

Ondetti MA, Pluscec J, Sabo EF, Sheehan JT, Williams N (1970) Synthesis of cholecystokinin-pancreozymin. I. The C-terminal dodecapeptide. Journal of the American Chemical Society 92:195-199.

Palkovits M (2003) Hypothalamic regulation of food intake. Ideggyogyaszati szemle 56:288-302.

Paxinos G, Watson C (2006) The rat brain in stereotaxic coordinates, sixth ed. Academic, New York.

Peters JH, Ritter RC, Simasko SM (2006) Leptin and CCK selectively activate vagal afferent neurons innervating the stomach and duodenum. American Journal of Physiology Regulatory; Integrative and Comparative Physiology 290:R1544-1549.

Petervari E, Ember A, Gobel G, Pakai E, Szekely M (2004) Signaling postprandial hyperthermia: a role for cholecystokinin. Journal of Thermal Biology 29:797-803.

Petervari E, Garami A, Pakai E, Szekely M (2005) Effects of perineural capsaicin treatment of the abdominal vagus on endotoxin fever and on a non-febrile thermoregulatory event. Journal of Endotoxin Research 11:260-266.

Petervari E, Garami A, Soos S, Szekely M, Balasko M (2010) Age-dependence of alpha-MSH-induced anorexia. Neuropeptides 44:315-322.

Petervari E, Miko A, Brunner S, Balasko M, Kofler B (2013) Thermoregulatory effects of alarin, Galanin meeting, Neuroscience Conference, San Diego.

Petervari E, Rostas I, Soos S, Tenk J, Miko A, Furedi N, Szekely M, Balasko M (2014) Age versus nutritional state in the development of central leptin resistance. Peptides 56:59-67.

Petervari E, Soos S, Szekely M, Balasko M (2011a) Alterations in the peptidergic regulation of energy balance in the course of aging. Current Protein & Peptide Science 12:316-324.

Petervari E, Szabad AO, Soos S, Garami A, Szekely M, Balasko M (2011b) Central alpha-MSH infusion in rats: disparate anorexic vs. metabolic changes with aging. Regulatory Peptides 166:105-111.

Popp E, Schneider A, Vogel P, Teschendorf P, Bottiger BW (2007) Time course of the hypothermic response to continuously administered neurotensin. Neuropeptides 41:349-354.

Rauch I, Lundstrom L, Hell M, Sperl W, Kofler B (2007) Galanin message-associated peptide suppresses growth and the budded-to-hyphal-form transition of Candida albicans. Antimicrobial Agents and Chemotherapy 51:4167-4170.

Raybould HE (2007) Mechanisms of CCK signaling from gut to brain. Current Opinion in Pharmacology 7:570-574.

Rezayat M, Ravandeh N, Zarrindast MR (1999) Cholecystokinin and morphine-induced hypothermia. European Neuropsychopharmacology: The Journal of the European College of Neuropsychopharmacology 9:219-225.

Roberts SB, Fuss P, Heyman MB, Evans WJ, Tsay R, Rasmussen H, Fiatarone M, Cortiella J, Dallal GE, Young VR (1994) Control of food intake in older men. JAMA 272:1601-1606.

Romanovsky AA, Ivanov AI, Shimansky YP (2002) Selected contribution: ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality. Journal of Applied Physiology (1985) 92:2667-2679.

Romanovsky AA, Ivanov AI, Szekely M (2000) Neural route of pyrogen signaling to the brain. Clinical Infectious Diseases: an Official Publication of the Infectious Diseases Society of America 31 Suppl 5:S162-167.

Rostas I, Furedi N, Tenk J, Miko A, Solymar M, Soos S, Szekely M, Petervari E, Balasko M (2015) Age-related alterations in the central thermoregulatory responsiveness to alpha-MSH. Journal of Thermal Biology 49-50:9-15.

Rostas I, Tenk J, Miko A, Furedi N, Soos S, Solymar M, Lengyel A, Szekely M, Gaszner B, Feller D, Petervari E, Balasko M (2016) Age-related changes in acute central leptin effects on energy balance are promoted by obesity. Experimental Gerontology 85:118-127.

Santic R, Fenninger K, Graf K, Schneider R, Hauser-Kronberger C, Schilling FH, Kogner P, Ratschek M, Jones N, Sperl W, Kofler B (2006) Gangliocytes in neuroblastic tumors express alarin, a novel peptide derived by differential splicing of the galanin-like peptide gene. Journal of Molecular Neuroscience 29:145-152.

Santic R, Schmidhuber SM, Lang R, Rauch I, Voglas E, Eberhard N, Bauer JW, Brain SD, Kofler B (2007) Alarin is a vasoactive peptide. Proceedings of the National Academy of Sciences of the United States of America 104:10217-10222.

Scarpace PJ, Matheny M, Shek EW (2000) Impaired leptin signal transduction with age-related obesity. Neuropharmacology 39:1872-1879.

Scarpace PJ, Tumer N (2001) Peripheral and hypothalamic leptin resistance with age-related obesity. Physiology & Behavior 74:721-727.

Schick RR, Harty GJ, Yaksh TL, Go VL (1990) Sites in the brain at which cholecystokinin octapeptide (CCK-8) acts to suppress feeding in rats: a mapping study. Neuropharmacology 29:109-118.

Schick RR, Schusdziarra V, Yaksh TL, Go VL (1994) Brain regions where cholecystokinin exerts its effect on satiety. Annals of the New York Academy of Sciences 713:242-254.

Schrodl F, Trost A, Strohmaier C, Bogner B, Runge C, Kaser-Eichberger A, Eberhard N, Santic R, Kofler B, Reitsamer HA (2013) Distribution of the regulatory peptide alarin in the eye of various species. Experimental Eye Research 106:74-81.

Schwartz GJ, Moran TH (1996) Sub-diaphragmatic vagal afferent integration of meal-related gastrointestinal signals. Neuroscience and Biobehavioral Reviews 20:47-56.

Serra-Prat M, Palomera E, Clave P, Puig-Domingo M (2009) Effect of age and frailty on ghrelin and cholecystokinin responses to a meal test. The American Journal of Clinical Nutrition 89:1410-1417.

Shiba K, Kageyama H, Takenoya F, Shioda S (2010) Galanin-like peptide and the regulation of feeding behavior and energy metabolism. The FEBS journal 277:5006-5013.

Shiraishi T (1990) CCK as a central satiety factor: behavioral and electrophysiological evidence. Physiology & Behavior 48:879-885.

Smith G, Gibbs J (1998) The satiating effect of cholecystokinin and bombesin-like peptides. G. Smith (Ed.). Satiation: From Gut to Brain, Oxford University Press, New York, 97-125.

Smith GP, Jerome C, Norgren R (1985) Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. The American Journal of Physiology 249:R638-641.

Soos S, Balasko M, Jech-Mihalffy A, Szekely M, Petervari E (2010) Anorexic vs. metabolic effects of central leptin infusion in rats of various ages and nutritional states. Journal of Molecular Neuroscience 41:97-104.

Soos S, Petervari E, Szekely M, Jech-Mihalffy A, Balasko M (2011) Complex catabolic effects of central alpha-MSH infusion in rats of altered nutritional states: differences from leptin. Journal of Molecular Neuroscience 43:209-216.

South EH, Ritter RC (1988) Capsaicin application to central or peripheral vagal fibers attenuates CCK satiety. Peptides 9:601-612.

Szekely M, Balasko M, Soos S, Petervari E (2012) Peptidergic regulation of food intake: changes related to age and body composition, in: Morrison, J.L. (Ed.), Food intake: Regulation, Assessing and Controlling. Nova Science Publishers Inc., New York, 83–104.

Szekely M, Petervari E, Balasko M (2010) Thermoregulation, energy balance, regulatory peptides: recent developments. Frontiers in bioscience 2:1009-1046.

Szekely M, Petervari E, Pakai E, Hummel Z, Szelenyi Z (2005) Acute, subacute and chronic effects of central neuropeptide Y on energy balance in rats. Neuropeptides 39:103-115.

Szekely M, Szelenyi Z (1979) Endotoxin fever in the rat. Acta physiologica Academiae Scientiarum Hungaricae 53:265-277.

Szekely M, Szelenyi Z (2005) Regulation of energy balance by peptides: a review. Current Protein & Peptide Science 6:327-353.

Szekely M, Szelenyi Z, Balasko M (1994) Cholecystokinin participates in the mediation of fever. Pflugers Archiv: European Journal of Physiology 428:671-673.

Szekely M, Szelenyi Z, Sumegi I (1973) Brown adipose tissue as a source of heat during pyrogen-induced fever. Acta physiologica Academiae Scientiarum Hungaricae 43:85-88.

Szelenyi Z, Bartho L, Szekely M, Romanovsky AA (1994) Cholecystokinin octapeptide (CCK-8) injected into a cerebral ventricle induces a fever-like thermoregulatory response mediated by type B CCK-receptors in the rat. Brain Research 638:69-77.

Tatemoto K, Rokaeus A, Jornvall H, McDonald TJ, Mutt V (1983) Galanin - a novel biologically active peptide from porcine intestine. FEBS letters 164:124-128.

Torregrossa AM, Smith GP (2003) Two effects of high-fat diets on the satiating potency of cholecystokinin-8. Physiology & Behavior 78:19-25.

Tsushima H, Mori M, Fujiwara N, Moriyama A (2003) Pharmacological characteristics of bombesin receptor mediating hypothermia in the central nervous system of rats. Brain Research 969:88-94.

Van Der Kolk N, Madison FN, Mohr M, Eberhard N, Kofler B, Fraley GS (2010) Alarin stimulates food intake in male rats and LH secretion in castrated male rats. Neuropeptides 44:333-340.

Wada A, Wong PF, Hojo H, Hasegawa M, Ichinose A, Llanes R, Kubo Y, Senba M, Ichinose Y (2013) Alarin but not its alternative-splicing form, GALP (Galanin-like peptide) has antimicrobial activity. Biochemical and Biophysical Research Communications 434:223-227.

Walsh JH, Lamers CB, Valenzuela JE (1982) Cholecystokinin-octapeptidelike immunoreactivity in human plasma. Gastroenterology 82:438-444.

Wang H, Wong PT, Spiess J, Zhu YZ (2005) Cholecystokinin-2 (CCK2) receptor-mediated anxiety-like behaviors in rats. Neuroscience and Biobehavioral Reviews 29:1361-1373.

Wang M, Chen Q, Li M, Zhou W, Ma T, Wang Y, Gu S (2014) Alarin-induced antidepressant-like effects and their relationship with hypothalamus-pituitary-adrenal axis activity and brain derived neurotrophic factor levels in mice. Peptides 56:163-172.

Wang M, Zhou W, Zhou X, Zhuang F, Chen Q, Li M, Ma T, Gu S (2015) Antidepressant-like effects of alarin produced by activation of TrkB receptor signaling pathways in chronic stress mice. Behavioural Brain Research 280:128-140.

Wank SA (1995) Cholecystokinin receptors. The American Journal of Physiology 269:G628-646.

Wank SA, Pisegna JR, de Weerth A (1994) Cholecystokinin receptor family. Molecular cloning, structure, and functional expression in rat, guinea pig, and human. Annals of the New York Academy of Sciences 713:49-66.

Webling KE, Runesson J, Bartfai T, Langel U (2012) Galanin receptors and ligands. Frontiers in Endocrinology 3:146.

Weiland TJ, Voudouris NJ, Kent S (2007) CCK(2) receptor nullification attenuates lipopolysaccharide-induced sickness behavior. American Journal of Physiology Regulatory; Integrative and Comparative Physiology 292:R112-123.

Werner MF, Souza GE, Zampronio AR (2006) Nimesulide-induced antipyresis in rats involves both cyclooxygenase-dependent and independent mechanisms. European Journal of Pharmacology 543:181-189.

West DB, Fey D, Woods SC (1984) Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. The American Journal of Physiology 246:R776-787.

WHO (2014) World Health Organization Ageing and health, Fact sheet N°404.

WHO (2016) World Health Organization Obesity and overweight. Fact sheet.

Winkels RM, Jolink-Stoppelenburg A, de Graaf K, Siebelink E, Mars M, de Groot L (2011) Energy intake compensation after 3 weeks of restricted energy intake in young and elderly men. Journal of the American Medical Directors Association 12:277-286.

Xu XJ, Andell S, Hao JX, Wiesenfeld-Hallin Z, Bartfai T (1995) The effects of intrathecal galanin message-associated peptide (GMAP) on the flexor reflex in rats. Regulatory Peptides 58:19-24.

Yoshimatsu H, Egawa M, Bray GA (1992) Effects of cholecystokinin on sympathetic activity to interscapular brown adipose tissue. Brain Research 597:298-303.

Zhuang F, Li M, Gao X, Wang Y, Wang D, Ma X, Ma T, Gu S (2016) The antidepressant-like effect of alarin is related to TrkB-mTOR signaling and synaptic plasticity. Behavioural Brain Research 313:158-171.

Zuzel KA, Rose C, Schwartz JC (1985) Assessment of the role of "enkephalinase" in cholecystokinin inactivation. Neuroscience 15:149-158.

12 Publications, lectures and posters

Publications as a basis for the present thesis:

Articles:

1 Balaskó Márta, Rostás Ildikó, Füredi Nóra, **Mikó Alexandra**, Cséplő Péter, Koncsecsko-Gáspár Margit, Soós Szilvia, Székely Miklós, Pétervári Erika: Age and nutritional state influence the effects of cholecystokinin on energy balance, Experimental Gerontology, 48: 11, 2013, 1180–1188 IF: 3.529

2 **Mikó Alexandra**, Balla Péter, Tenk Judit, Balaskó Márta, Soós Szilvia, Székely Miklós, Brunner Susanna, Kofler Barbara, Pétervári Erika: Thermoregulatory effect of alarin, a new member of the galanin peptide family, 2014, Temperature 1:1, 1–6.

3. **Mikó Alexandra,** Füredi Nóra, Tenk Judit, Rostás Ildikó, Soós Szilvia, Solymár Margit, Székely Miklós, Balaskó Márta, Brunner Susanne, Kofler Barbara, Pétervári Erika: Acute central effects of alarin on the regulation on energy homeostasis, Neuropeptides. 2016, S0143-4179(16)30087-7. IF: 2.726

IF: 6.255 of thesis related publications

Cumulative IF: 39.687 (with citable abstracts)

Citable abstracts

1. **Mikó A**, Füredi N, Rostás I: Regulation of energy balance: the role of cholecystokinin in function of age and nutritional state. Acta medica marisisensis 61 (S7): 34 (2015)
2. Füredi N, Gebhardt H, **Mikó A**, Soós Sz, Balaskó M, Székely M, Pétervári E : Obesity-induced shift in the age-related alterations of CCK. OBESITOLOGIA HUNGARICA 14:(Suppl. 2) p. 51. (2015)
3. **Mikó A**, Kéringer P, Füredi N, Soós Sz, Balaskó M, Brunner S, Kofler B, Pétervári E: The potential role of alarin, a novel peptide mediator in body weight regulation OBESITOLOGIA HUNGARICA 14:(Suppl. 2) p. 41. (2015)
4. **Mikó A**., Balla P., Aubrecht B., Füredi N., Soós Sz., Székely M., Balaskó M., Brunner S., Kofler B., Pétervári E.: The potential contribution of alarin to the regulation of energy balance in rats, Joint meeting of the Federation of European

Physiological Societies (FEPS) and the Hungarian Physiological Society, 27-30 August 2014, Budapest, Acta Physiologica, 2014; (211), 697, 127-127

5. Petervári E., Füredi N., **Mikó A.,** Heppeler G., Gáspár-Koncsecskó M., Soós Sz., Székely M., Balaskó M.: Age related disparate shifts in the anorexic and hyperthermic effects of cholecystokinin (CCK): The influence of body composition, The Eleventh International Symposium of Neurobiology and Neuroendocrinilogy of Aging, Bregenz, 2012. 07.29-08.03. Experimental Gerontology, Volume 48, Issue 7, Pages 593-700. (July 2013) IF: 3.911 (2012)
6. Füredi N., **Mikó A.,** Heppeler GAI., Szekeres-Solymár M., Balaskó M., Soós S., Székely M., Pétervári E.: Disparate age-associated alterations in cholecystokinin effects on energy homeostasis. Magyar Idegtudományi Társaság IBRO International Workshop Pécs, 2012. 01. 19-21. CLIN. NEUROSCI./Ideggyógyászati Szemle 65 (S1): 22 (2012)

Other publications

1. Dóra Mosztbacher, Nelli Farkas, Péter Hegyi, Margit Solymár, Gabriella Pár, Judit Bajor, Ákos Szücs, József Czimmer, Katalin Márta, Alexandra Mikó, Zoltán Rumbus, Péter Varjú, Andrea Párniczky: Restoration of energy level in the early phase of acute pediatric pancreatitis. Accepted in World Journal of Gastroenterology, IF: 2.787
2. Zoltan Rumbus, Robert Matics, Peter Hegyi, Csaba Zsiboras, Imre Szabo, Anita, Illes, Erika Petervari, Marta Balasko, Katalin Marta, **Alexandra Miko**, Andrea Parniczky, Judit Tenk, Ildiko Rostas, Margit Solymar, Andras Garami: Fever Is Associated with Reduced, Hypothermia with Increased Mortality in Septic Patients: a Meta-Analysis of Clinical Trials, accepted in PlosOne, IF: 3.54
3. Tenk J, Mátrai P, Hegyi P, Rostás I, Garami A, Szabó I, Solymár M, Pétervári E, Czimmer J, Márta K, **Mikó A**, Füredi N, Párniczky A, Zsiborás C, Balaskó M.: In Obesity, HPA Axis Activity Does Not Increase with BMI, but Declines with Aging: A Meta-Analysis of Clinical Studies. PLoS One. 2016 Nov 21;11(11):e0166842. IF: 3.54
4. Zsiborás C, Mátics R, Hegyi P, Balaskó M, Pétervári E, Szabó I, Sarlós P, **Mikó A**, Tenk J, Rostás I, Pécsi D, Garami A, Rumbus Z, Huszár O, Solymár M: Capsaicin and capsiate could be appropriate agents for treatment of obesity: a

meta-analysis of human studies. Crit Rev Food Sci Nutr. 2016 Dec 21:0. doi: 10.1080/10408398.2016.1262324. IF: 5.492

5. Tenk J., Rostás I., Füredi N., **Mikó A.,** Soós Sz., Solymár M., Gaszner B., Székely M., Pétervári E., Balaskó M: Acute central effects of corticotropin-releasing factor (CRF) on energy balance: effects of age and gender, Peptides. 2016 Nov;85:63-72. IF: 2.535
6. Tenk J., Rostás I., Füredi N., **Mikó A.,** Solymár M., Soós Sz., Gaszner B., Székely M., Pétervári E., Balaskó M.: Age-related changes in central effects of corticotropin-releasing factor (CRF) suggest a role for this mediator in aging anorexia and cachexia, Accepted in AGE, IF: 2.5
7. Rostás I., Tenk J., **Mikó A.,** Füredi N., Soós Sz., Solymár M., Lengyel A., Székely M., Pétervári E., Balaskó M.: Age-related changes in acute central leptin effects on energy balance are promoted by obesity, Exp Gerontol. 2016;85:118-127. IF: 3.35
8. Nora Furedi, **Alexandra Miko**, Bianka Aubrecht, Balazs Gaszner, Diana Feller, Ildiko Rostas, Judit Tenk, Szilvia Soos, Marta Balasko, Andras Balogh, Marianna Pap, Erika Petervari: Regulatory alteration of energy homeostasis in spontaneously hypertensive rats (SHR). J Mol Neurosci. 2016;59(4):521-30. IF: 2.352
9. Rostás Ildikó, Füredi Nóra, Tenk Judit, **Mikó Alexandra**, Solymár Margit, Soós Szilvia, Székely Miklós, Pétervári Erika, Balaskó Márta: Age-related alterations in the central thermoregulatory responsiveness to alpha-MSH, Journal of Thermal Biology: Environment Evolution and Medicine, 2015; 49-50:9-15. IF: 1.544
10. Pétervári Erika, Rostás Ildikó, Soós Szilvia, Tenk Judit, **Mikó Alexandra**, Füredi Nóra, Székely Miklós, Balaskó Márta: Age versus nutritional state in the development of leptin resistance, 2014; Peptides 56C:59-67 IF: 2.522
11. Nagy Ágnes, Losonczy Hajna, Tóth Orsolya, Kosztolányi Szabolcs, **Mikó Alexandra**, Mózes Réka, Dávid Marianna: Masszív vérzés kezelése magas titerű inhibítoros haemophiliás betegnél, szekvenciális áthidaló kezeléssel. Hematológia-Transzfúziológia, 2011; 44: 148-151.

Other citable abstracts

1. Kéringer P, **Mikó A**, Tékus É, Füredi N, Balaskó M, Pétervári E.: Different ways of body composition measurements in rats, OBESITOLOGIA HUNGARICA 14:(Suppl. 2) p. 52. (2015)
2. Füredi N., Aubrecht B., Balla P., **Mikó A.,** Soós Sz., Székely M., Balaskó M., Gaszner B., Pétervári E.: The role of the melanocortin system and neuropeptide Y in the regulation of energy homeostasis in SHR rats, Joint meeting of the Federation of European Physiological Societies (FEPS) and the Hungarian Physiological Society, 27-30 August 2014, Budapest, Acta Physiologica, 2014; (211), 697, 154-154
3. Rostas I, Tenk J, **Mikó A**, Furedi N: Complex effects of alpha-melanocyte-stimulating-hormone during the course of aging. Periodicum Biologorum 115 (S2): 50 (2013), IF: 0.180
4. **Mikó A.,** Füredi N., Párniczky A., Szekeres-Solymár M., Gáspár-Koncsecskó M., Soós S., Pétervári E., Székely M., Balaskó M.: Perinatal capsaicin-desensitization alters energy homeostasis in rats. Magyar Idegtudományi Társaság IBRO International Workshop Pécs, 2012. 01. 19-21. CLIN. NEUROSCI./ Ideggyógyászati Szemle 65 (S1): 45 (2012)
5. Balaskó M., Füredi N., **Mikó A.,** Solymár M. Soós Sz. Székely M. Pétervári E.: A hypertonia, az energiaháztartás, illetve a melanocortin rendszer összefüggése patkányban, A Magyar Hipertónia Társaság X. Nemzetközi Továbbképző Kurzusa és XIX. Kongresszusa, 2011.november 30-decebmer 3., HYPERTONIA ÉS NEPHROLOGIA 15 (Suppl. 3): S29 (2011)
6. N. Füredi, **A. Mikó**, A. Párniczky, E. Pétervári and S. Soós (2011): Central melanocortin responsiveness in spontaneously hypertensive rats (SHR). A Magyar Idegtudományi Társaság XIII. Konferenciája, Frontiers in Neuroscience. Conference Abstract: IBRO International Workshop 2011.doi:10.3389/conf.fnins.2011.84.00123
7. **Mikó A.**, Füredi N.: Soós S., Balaskó M., Pétervári E., Székely M. (2011): Alteration of energy homeostasis in spontaneously hypertensive rats (SHR). A Magyar Élettani Társaság LXXV. Vándorgyűlése, a Magyar Anatómus Társaság XVI. Kongresszusa, a Magyar Kísérletes és Klinikai Farmakológiai Társaság Experimentális Szekciója és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság közös tudományos konferenciája, Acta Physiologica 2011; Volume 202, Supplement 684:P59, 79-80. IF: 3.09

Lectures, posters related to the thesis

1. **Mikó Alexandra**, Klespitz Péter, Sohonyay Fanni Mirella, Füredi Nóra, Susanne Brunner, Barbara Kofler, Balaskó Márta, Pétervári Erika: Prosztaglandinok mediálják-e az alarin lázszerű hőszabályozási hatását? FAMÉ, Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája, Pécs, 2016. június 1-4.

2. **Mikó A.**, Füredi N. Rostás I.: Regulation of energy balance: the role of cholecystokinin in function of age and nutritional state, The 8th Conference of PhD Students and the 5th Conference of Post-Dos researchers in Medicine and Pharmacy, Marosvásárhely, 2015. December 9-10.
3. **Mikó A.**, Kéringer P., Füredi N., Soós Sz., Balaskó M., Brunner S., Kofler B., Pétervári E.: The potential role of alarin, a novel peptide mediator in body weight regulation, 5th Central European Congress on Obesity, 2015 október 1-3., Budapest
4. **Mikó A**., Balla P.,Tenk J., Balaskó M., Soós Sz., Brunner S., Kofler B., Pétervári E.: Thermoregulatory effects of alarin, a new member of the galanin peptide family, Neuropeptides 2015 szept. 28-október 1., Aberdeen
5. **Mikó A.**, Balla P., Kéringer P., Füredi N., Sohonyay F., Pétervári E.: Az alarin egy új katabolikus neuropeptid? A Magyar Kísérletes és Klinikai Farmakológiai Társaság Experimentális Farmakológia szekciójának IX. szimpóziuma, 2015.március 26-28. Velence
6. **Mikó. A.**, Balla P., Aubrecht B., Füredi N., Balaskó M., Brunner S., Kofler B., Pétervári E.: Effects of alarin on the regulation of energy balance in rats, YES (Young European Scientist) Meeting, 2014. szeptember 18-21. Porto, Portugal
7. **Mikó A.**, Balla P., Aubrecht B., Füredi N.1, Soós S., Székely M., Balaskó M, Brunner S., Kofler B., Pétervári E.: The potential contribution of alarin to the regulation of energy balance in rats, Joint meeting of the Federation of European Physiological Societies (FEPS) and the Hungarian Physiological Society, 27-30 August 2014, Budapest
8. **Mikó A.,** Füredi N., Rostás I.: Mechanisms of sarcopenic obesity: the role of body composition in the development of age-related peptidergic regulatory changes, Global Awareness Society International 23th Conference, 2014. május 23-27. Montego bay, Jamaica
9. **Mikó A.**, Balla P., Füredi N., Balaskó M., Brunner S., Kofler B., Pétervári E.: Effects of alarin on the thermoregulation of rats, IBRO Workshop, Debrecen, 2014. január 16-17.
10. Balasko M., **Mikó A.,** Füredi N., Soos. Sz., Brunner. S., Kofler B., Petervari E.: Food intake-related effect of alarin, a new member of the galanin peptide family, Neuropeptides 2015 szept.28-október 1., Aberdeen
11. Balasko M., **Mikó A.,** Rostas I., Soos. Sz., Brunner. S., Kofler B., Petervari E.: Alarin: a new catabolic neuropeptide, Neuropeptides 2015 szept.28-október 1., Aberdeen
12. Pétervari E., **Miko A**., Brunner S., Balasko M., Kofler B.: Thermoregulatory effects of alarin, Galanin meating, Neuroscience 2013 conference, San Diego
13. Rostás I., **Mikó A**., Soós Sz., Balaskó M., Pétervári E., Székely M.: Centrális cholecystokinin (CCK) receptorok korfüggő energetikai hatásai Egymást Értő Szakemberek Nemzetközi Konferencia, Budapest, 2013. 10. 1-2.
14. Füredi N, **Mikó A.**, Tenk J.: A cholecystokinin kor- és testösszetétel függő anorexigén hatásának vizsgálata TDK konferencia, 2013. február 7-8.

15. Füredi N., Gebhardt H., **Mikó A.**, Soós Sz., Balaskó M., Székely M., Pétervári E.: Obesity-induced shift in the age-related alterations of CCK, 5th Central European Congress on Obesity, 2015 október 1-3., Budapest
16. Füredi N, TenkJ., **Mikó A.**, Rostás I.: Cholecystokinin effects on energy balance depend on age. LIMSC 2013. március 13-17.
17. Petervári E., Füredi N., **Mikó A**.,Heppeler G., Gáspár-Koncsecskó M., Soós Sz., Székely M., Balaskó M.: Age related disparate shifts in the anorexic and hyperthermic effects of cholecystokinin (CCK): The influence of body composition, The Eleventh International Symposium of Neurobiology and Neuroendocrinilogy of Aging, Bregenz, 2012. 07.29-08.03.
18. Füredi N., Tenk J., **Mikó A.**: Age-associated alterations in cholecystokinin effects conserning energy balance are disparated, 8th Croatian Student Submit, Zágráb, 2012.03.28-31.
19. Füredi N., Rostás I., Tenk J., **Mikó A.**, Balaskó M.: Cholecystokinin effects on energy balance depend on age and body composition, YES(Young European Scientist) Meeting, 2014. szeptember 18-21. Porto, Portugal
20. Füredi N., Rostás I., Tenk J., **Mikó A.**, Balaskó M.: Cholecystokinin effects on energy balance depend on age and body composition, AIMS Meeting, 7-9 March 2014. Lisbon, Portugal
21. Balla Péter, Aubrecht Bianka, Füredi Nóra, **Mikó Alexandra**: Az alarin hatásai az energiaháztartás szabályozásában
22. Füredi N., Rostás I., Tenk J., **Mikó A**.: Cholecystokinin effects on energy balance depend on age and body composition. 9. Szentágotai János Interdiszciplináris Konferencia, Pécs, 2013.05. 3-4.
23. Füredi N., Rostás I., Tenk J., **Mikó A.**: A cholecystokinin (CCK) anorexigén hatásának vizsgálata: az életkor és a testösszetétel befolyásoló hatása V. Nemzetközi XI. Országos Interdiszciplináris Grastyán Konferencia, 2013. április 17-19.
24. Füredi N. Rostás I., Tenk J., **Mikó A.**, Pétervári E., Balaskó M.: cholecystokinin effects on energy balance depend on age and body composition, the Student Scientific Conference on Biotechnology and Biomedicine, Brno, 2013. április 10-12.

Other posters and lectures

1. **Alexandra Mikó**, Margit Solymár, Eszter Pákai, Nóra Kovács, Zoltán Rumbus, Csaba Zsiborás, András Garami **:** The role of the transient receptor potential vanilloid-1 channel and regulatory neuropeptides in the age-related changes of body mass in TRPV1 desensitized rats, IBRO Workshop, 2016. január 20-21., Budapest
2. **Mikó A.**, Aubrecht B., Füredi N., Pétervári E.: Maladaptive response to fasting and dysregulation of satiety in spontaneously hypertensive rats, Third International Symposium on Hypertension, 2014. November 28-30. Eszék, Horvátország

3. **Mikó A.**: Tehetséggondozás az egyetemen: a Tudományos Diákkör, III**.** Interdiszciplinális Doktorandusz Konferencia 2014 - IDK2014 -Mandulavirágzási napok Pécs, 2014. április 15-17.
4. **Mikó. A.**, Füredi N., Gaszner B., Pétervári E.: Role of neuropeptides in the regulation of energy homeostasis in SHR rats, AIMS Meeting, 7-9 March 2014. Lisbon, Portugal
5. **Mikó A.**, Füredi N., Tenk J., Rostás I.: Changes in regulatory effects of neuropeptide Y, cholecystokinin and melanocortins on energy homeostasis in spontaneously hypertensive rats, 2nd International Doctoral Workshop on Natural Sciences, 2013. 09 11-12.
6. **Mikó A**., Nagy P., Koncsecskó M., Balaskó M., Székely M., Pétervári E., Soós Sz.: Nemi eltérések a korfüggő energetikai változásokban állatkísérletek alapján, Egymást Értő Szakemberek Nemzetközi Konferencia, Budapest, 2013. 10 1-2.
7. **Mikó A.**, Tenk J., Rostás I., Füredi N.: Táplálékfelvételt befolyásoló neuropeptidek hatásának vizsgálata spontán hipertenzív patkányokban, V. Nemzetközi XI. Országos Interdiszciplináris Grastyán Konferencia, 2013. április 17-19.
8. **Mikó A.,** Tenk J., Füredi N.: Orexigén és anorexigén neuropeptidek hatásának vizsgálata spontán hipertenzív patkányokban, OTDK konferencia, 2013. április 2-5.
9. **Mikó A.**, Rostás I., Tenk J., Füredi N.: Spontaneously hypertensive rats (SHR) show complex changes in their energy homeostasis, LIMSC, 2013. március 13-17.
8. **Mikó A.,** Tenk J., Füredi N.: Orexigén és anorexigén neuropeptidek hatásának vizsgálata spontán hipertenzív patkányokban TDK konferencia, 2013. február 7-8.
9. **Mikó A.:** Hematológia a mindennapi ember számára. A Tudomány Ünnepe alkalmából megrendezett Konferencia, 2012. november 22.
10. **Mikó A.**, Füredi N., Rostás I., Tenk J.: Metabolic adaptation to food deprivation in spontaneously hypertensive rats. János Szentágothai Memorial Conference and Student Competition 2012.október 29-30.
11. **Mikó A.,** Füredi N. Tenk J.: Complex changes in the energy homeostasis of spontaneously hypertensive rats (SHR), 8th Croatian Student Submit, Zágráb, 2012.03.28-31.
10. **Mikó A.**, Füredi N., Párniczky A., Szekeres-Solymár M., Gáspár-Koncsecskó M., Soós S., Pétervári E., Székely M., Balaskó M.: Perinatal capsaicin-desensitization alters energy homeostasis in rats. Magyar Idegtudományi Társaság IBRO International Workshop Pécs, 2012. 01. 19-21.
11. **Mikó A.,** Füredi N**. :** Spontán hipertenzív patkányok energia-homeosztázisának vizsgálata: éhezés, újratáplálás és melanokortin hatása, Minősítő Konferencia, 2011. okt. 6-7.
12. **Mikó A.,** Füredi N**.** Spontán hipertenzív patkányok éhezésre és újratáplálásra adott válasza, Balatonfüredi HMAA Konferencia, 2011. aug. 19-20.
13. **Mikó A**., Füredi N.: Soós S., Balaskó M., Pétervári E., Székely M. (2011): Alteration of energy homeostasis in spontaneously hypertensive rats (SHR). A Magyar Élettani Társaság LXXV. Vándorgyűlése, a Magyar Anatómus Társaság XVI. Kongresszusa, a Magyar Kísérletes és Klinikai Farmakológiai Társaság

Experimentális Szekciója és a Magyar Mikrocirkulációs és Vaszkuláris Biológiai Társaság közös tudományos konferenciája, 2011. június 8-11.

14. **Mikó A.,** Füredi N**. :** Spontán hipertenzív patkányok éhezéshez és újratápláláshoz való energetikai adaptációja, Korányi Frigyes Szakkollégium által szervezett konferencia, 2011. április 14-15.
15. **Mikó A.,** Füredi N**.** : Éheztetés és újratáplálás hatása spontán hipertenzív patkányban, III. Nemzetközi IX. Országos Interdiszciplináris Grastyán Konferencia, 2011.03.29.-04.01
16. **Mikó A.,** Füredi N**. :** Éhezés és újratáplálás spontán hipertenzív patkányokban, XVIII. Marosvásárhelyi TDK-konferencia 2011. 03. 17-20.
17. **Mikó A.,** Füredi N**. :** Spontán hipertenzív patkányok energia háztartásának vizsgálata: éhezéshez és újratápláláshoz való adaptáció, PTE-ÁOK Tudományos Diákköri Konferencia, 2011. 02. 17-18., Pécs
18. **Mikó A.,** Füredi N**.** : Spontán hipertenzív patkányok energia háztartásának vizsgálata: éhezéshez való adaptáció, Grastyán Endre Szakkollégium által szervezett IV. Intézményi Tudományos Konferencia, 2010. 12. 2-3.
19. **Mikó A.,** Füredi N**. :** Magas vérnyomásos patkányok testtömeg csökkenésre adott válasza, Grastyán Endre Szakkollégium által szervezett, a Magyar Tudomány Ünnepe alkalmából rendezett Tudományos Ismeretterjesztő Konferencia, 2010. november 24-25.
20. Solymár Margit**, Mikó Alexandra** , Füredi Nóra, Victor Obomate Tobins, Kéringer Patrik, Khidhir Nóra, Zsiborás Csaba, Rumbus Zoltán , Garami András: A Tranziens Receptor Potenciál Ankyrin-1 (TRPA1) csatorna szerepe hőterhelésben és endotoxin által kiváltott szisztémás gyulladásban, FAMÉ, Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája, Pécs, 2016. június 1-4.
21. 26 Kéringer P., **Mikó A.,** Tékus É., Füredi N., Balaskó M., Pétervári E.: Different ways of body composition measurements in rats, 5th Central European Congress on Obesity, 2015 október 1-3., Budapest
22. Füredi N., **Mikó A.**, Gaszner B., Tenk J., Rostás I., Soós Sz., Balaskó M., Pétervári E.: Dysregulation of energy balance in spontaneously hypertensive rats: the melanocortin and neuropeptide Y system
23. Rostás I., **Mikó A.**, Füredi N., Tenk J.: Az alpha-melanocyta-stimuláló-hormon szerepe az energiaháztartás szabályozásában V. Nemzetközi XI. Országos Interdiszciplináris Grastyán Konferencia, 2013. április 17-19.
24. Füredi N, **Mikó A**., Tenk J.: A cholecystokinin kor- és testösszetétel függő anorexigén hatásának vizsgálata OTDK konferencia, 2013. április 2-5.
25. Füredi N., **Mikó A**., Tenk J., Rostás I.: Effects of neuropeptides in spontaneously hypertensive rats 9th International Biomedical Croatian Student Summit, 2013. március 20-23.
26. Rostás I., **Mikó A.**, Füredi N., Tenk J.: Coplex effects of neoropeptide alpha-melanocyte stimulating-hormone on energy homeostasis during the course of aging, LIMSC 2013. március 13-17.

27. Füredi N., **Mikó A.**, Tenk J.: Copmlex changes in the energy homeostasis of spontaneously hypertensive rats (SHR) 22nd Annual BioCity Simpisium, Turku, 2012. augusztus 22-23.
28. Pétervári E., **Mikó A.**, Szekeres-Solymár M., Soós Sz., Székely M., Balaskó M.:Central effects of cholecystokinin (CCK) on metabolic/thermoregulatory parameters of the energy homeostasis during the course of aging, BPS Focused Meeting on Neuropeptides, London, 2012.06.7-9**.**
29. Tenk J., **Mikó A.**, Füredi N., Lőrincz O.: Age-assiciated alterations in corticotrophin effects on energy homeostasis are disparate, 8th Croatian Student Submit, Zágráb, 2012.03.28-31.
30. Füredi N., **Mikó A.**, Heppeler GAI., Szekeres-Solymár M., Balaskó M., Soós S., Székely M., Pétervári E.: Disparate age-associated alterations in cholecystokinin effects on energy homeostasis. Magyar Idegtudományi Társaság IBRO International Workshop Pécs, 2012. 01. 19-21.
31. Füredi N., **Mikó A.** : Effect of central melanocortin in spontaneously hypertensive rats (SHR).Hungarian Medical Association of America - HUNGARY CHAPTER – Balatonfüredi konferencia 2011. augusztus 19-20.
32. Füredi N., **Mikó A.** : Centrális melanocortin katabolikus hatása spontán hipertenzív patkányban (SHR). Korányi Frigyes Szakkollégium XVI. Tudományos Fóruma, 2011. április 14-15.
33. Füredi N., **Mikó A**.: Melanocortin hatása spontán hipertenzív patkányban . III. Nemzetközi IX. Országos Interdiszciplináris Grastyán Konferencia, 2011.03.29.-04.01.
34. Füredi N., **Mikó A**., A. Párniczky, E. Pétervári and S. Soós : Central melanocortin responsiveness in spontaneously hypertensive rats (SHR). Magyar Idegtudományi Társaság IBRO International Workshop Pécs, 2011. 01. 20-22.
35. Füredi N., **Mikó A**.: Az alpha-melanocyta stimuláló hormon (alpha-MSH) katabolikus hatásának vizsgálata spontán hipertenzív patkányokban. XVIII. Marosvásárhelyi TDK-konferencia, 2011. 03. 17-20.
36. Füredi N., **Mikó A**.: A katabolikus alpha-melanocyta stimuláló hormon hatása spontán hipertenzív patkányban. PTE-ÁOK Tudományos Diákköri Konferencia, 2011. 02.17-18., Pécs
37. Füredi N., **Mikó A**.: Spontán hipertenzív patkányok energiaháztartásának vizsgálata centrálisan alkalmazott melanocortin agonistára adott válaszkészség Grastyán Endre Szakkollégium által szervezett IV. Intézményi Tudományos Konferencia, 2010. 12. 2-3.
38. Dr. Soós S., **Mikó A.**, Füredi N., Dr. Balaskó M., Dr. Székely M., Dr. Pétervári E.: Tápláltsági állapot befolyásacentrális Alpha-MSH infúzió complex energetikai hatásaira A Magyar Gerontológiai és Geriátriai Társaság XXXIII. Kongresszusa, 2010. November 26-27.
39. Füredi N., **Mikó A.** : Spontán magasvérnyomásos patkányok energiaháztartásának szabályozása Grastyán Endre Szakkollégium által szervezett, a Magyar Tudomány Ünnepe alkalmából rendezett Tudományos Ismeretterjesztő Konferencia, 2010. november 24-25.

40. Khidhir Nóra, Solymár Margit, **Mikó Alexandra**, Füredi Nóra, Pákai Eszter, Kéringer Patrik, Kovács Nóra, Rumbus Zoltán, Zsiborás Csaba, Garami András: A tranziens receptor potenciál vanilloid-1 csatorna és katabolikus neuropeptidek szerepe a korfüggő testtömeg változások kialakulásában patkányokban, FAMÉ, Magyar Farmakológiai, Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája, Pécs, 2016. június 1-4.
41. Balaskó M, Rostás I., **Mikó A.**, Füredi N., Tenk J., Soós S., Székely M., Pétervári E.: Age-related shifts in the acute anorexic effects of leptin: the influence of body composition, 15th Meeting of the Hungarian Neuroscience Society, 2015. január 22-23., Budapest
42. Füredi N., Aubrecht B., **Mikó A.**, Gaszner B., Pétervári E.: Melanocortin system in the regulation of energy homeostasis in SHR rats, IBRO Workshop, Debrecen, 2014. január 16-17.
43. Balasko M., Tenk J., **Miko A.**, Gaspar-Koncsecskó M., Soos Sz., Szekely M., Peretvari E.: Age-associated alterations int he acute effects of corticotropin-releasing factor (CRF) on energy balance, 3rd Congress of Croatian Physiological Society, 2013. 09. 13-15.
44. Rostás I., Tenk J., **Mikó A.**, Füredi N.: Complex effect of alpha-melanocyte-stimulating hormone during thecourse of aging, 3rd Congress of Croatian Physiological Society, 2013. 09. 13-15.
45. Rostás I., Tenk J., **Mikó A.**, Füredi N.: Complex effects of alpha-MSH during the course of aging, 2nd International Doctoral Workshop on Natural Sciences, 2013. 09 11-12.
46. Tenk J., Rostás I., **Mikó A.**, Füredi N.: Age-associated alterations in acute corticotropin effects on energy homeostasis, 2nd International Doctoral Workshop on Natural Sciences, 2013. 09 11-12.
47. Petervari E., Furedi N., **Miko A**., Mihalffy-Jech A., Soos S., Szekely M., Balasko M.: Effects of anorexigenic and orexigenic neuropeptides in spontaneously hypertensive rats. 2013.05-29-06.01. Gdynia, Poland
48. Tenk J., Füredi N., **Mikó A.**, Rostás I.: A corticotropin-releasing factor (CRF) akut életkorfüggő hatásai az energia háztartásban V. Nemzetközi XI. Országos Interdiszciplináris Grastyán Konferencia, 2013. április 17-19.
49. Tenk J., Füredi N., **Mikó A**.: Korfüggő eltérések a corticotropin-releasing factor (CRF) akut centrális energetikai hatásaiban OTDK konferencia, 2013. április 2-5.
50. Tenk J., Füredi N., **Mikó A**.: Korfüggő eltérések a corticotropin-releasing factor (CRF) akut centrális energetikai hatásaiban TDK konferencia, 2013. február 7-8.
51. Balaskó M., Füredi N., **Mikó A**. Solymár M. Soós Sz. Székely M. Pétervári E.: A hypertonia, az energiaháztartás, illetve a melanocortin rendszer összefüggése patkányban, A Magyar Hipertónia Társaság X. Nemzetközi Továbbképző Kurzusa és XIX. Kongresszusa, 2011.november 30-decebmer 3.
52. Kéringer Patrik, Füredi Nóra, Khidhir Nóra, **Mikó Alexandra**, Solymár Margit, Gaszner Balázs , Garami András: Centrálisan adott cholecystokinin termoregulációs hatásának vizsgálata patkányban, FAMÉ, Magyar Farmakológiai,

Anatómus, Mikrocirkulációs és Élettani Társaságok Közös Tudományos Konferenciája, Pécs, 2016. június 1-4.

53. Füredi N., Aubrecht B., Gebhardt H., **Mikó A.**, Gaszner B., Balaskó M., Pétervári E.: A katabolikus hatású melanocortin rendszer és a neuropeptid Y lehetséges szerepe spontán hipertenzív patkányok energia-egyensúlyának regulációjában, A Magyar Kísérletes és Klinikai Farmakológiai Társaság Experimentális Farmakológia szekciójának IX. szimpóziuma, 2015.március 26-28. Velence
54. Füredi N., Gebhardt H., Aubrecht B., **Mikó A**., Gaszner B., Pétervári E.: Az alpha-melanocyta stimuláló hormon termelődésének és a melanocortin-4-es receptor expressziójának korfüggő változásai patkány hypothalamusban, A Magyar Kísérletes és Klinikai Farmakológiai Társaság Experimentális Farmakológia szekciójának IX. szimpóziuma, 2015.március 26-28. Velence
55. Nagy P., Novinszky P., Tenk J., **Mikó A**., Füredi N., Rostás I, Pétervári E., Balaskó M., Székely M., Soós S.: Changes of efficacy of the centrally applied alpha-melanocyte-stimulating hormone (alpha-MSH) or neuropeptide Y (NPY) and peripherally administered cholecystokinin (CCK) in male and female rats of various ages, 15th Meeting of the Hungarian Neuroscience Society, 2015. január 22-23., Budapest
56. Pétervári E., Balla P., Ehlers S., **Mikó A.**, Füredi N.: Melanocortins in age-related metabolic dysregulation: the influence of nutritional state, Third International Symposium on Hypertension, 2014. November 28-30. Eszék, Horvátország
57. Füredi N., Gebhardt H., Kéringer P., **Mikó A.**, Gaszner B., Pétervári E.: Dysregulation of energy balance in spontaneously hypertensive rats: the melanocortin and neuropeptide Y system, Third International Symposium on Hypertension, 2014. November 28-30. Eszék, Horvátország
58. Füredi N., Aubrecht B., Balla P., **Mikó A.**, Soós S., Székely M., Balaskó M., Gaszner B., Pétervári E.: The role of the melanocortin system and neuropeptide Y in the regulation of energy homeostasis in SHR rats, Joint meeting of the Federation of European Physiological Societies (FEPS) and the Hungarian Physiological Society, 27-30 August 2014, Budapest
59. Aubrecht Bianka, Balla Péter, Füredi Nóra, **Mikó Alexandra**: Melanokortin rendszer hatásának vizsgálata SHR patkányokban
60. Rostás I., Füredi N., Tenk J., **Mikó A.**: Thermoregulatory effects of alpha-melanocyte-stimulating hormone depend on age. János Szentágothai Memorial Conference and Student Competition 2012. október 29-30.
61. Novinszky P., Nagy P., Füredi N., Tenk J., Rostás I., **Mikó A.**, Pétervári E., Balaskó M., Székely M., Soós S.: Investigation of complex effects of central insulin infusion on energy balance in male Wistar rats of different ages, 15th Meeting of the Hungarian Neuroscience Society, 2015. január 22-23., Budapest