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

Ph.D. thesis

Alexandra Mikó M.D.

Head of Doctoral School: Prof. Erika Pintér M.D., Ph.D., D.Sc.

Head of the Doctoral Program: Prof. Erika Pintér M.D., Ph.D., D.Sc.

Supervisor: Erika Pétervári M.D., Ph.D.

INSTITUTE FOR TRANSLATIONAL MEDICINE

MEDICAL SCHOOL, UNIVERSITY OF PÉCS

Pécs, 2017

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 [1, 2].

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 [3]. 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 [4].

This thesis focuses on the role of two neuropeptides, **cholecystokinin (CCK)** and **alarin** in the regulation of energy balance.

1.1. Peptidergic regulation of the energy homeostasis and aging

Energy balance involves regulation of body temperature via heat production (metabolic rate, MR) and heat loss (HL), and that of body weight (BW) via food intake (FI) and MR. This can be described as a dynamic state.

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) [5]. In addition, in case of imbalance between MR and HL, the core body temperature (Tc) will change (hypothermia, hyperthermia).

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. 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 [6-10]. 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.

1.2 Cholecystokinin

Cholecystokinin is an anorexigenic brain-gut peptide [11], it inhibits the FI (after both peripheral and central administration), gastric movement and the production of gastric acid for a while, thus it has an important role in forwarding and digesting the consumed food and inducing satiety [12]. The latter effect indicates that CCK can influence cerebral functions related to energy balance. The biologically active form is an octapeptide (CCK-8) with o-sulfated tyrosin residue found also in mammals [13].

Two types of CCK receptors are known (type 1 and 2). Peripheral type-1 CCK receptors (CCK1R-s) are located mainly on the afferent fibers of the abdominal vagus [14, 15]. Some CCK1R-s have been detected also in the brain, e.g. in the nucleus of the solitary tract (NTS). The NTS serves as a portal for assessing and integrating visceral afferent signals (including CCK-related signals).

The type-2 receptors are located primarily in the central nervous system [16-19]. Therefore, in contrast to peripheral administration, centrally applied CCK acts mainly on CCK2R-s of hypothalamic and other nuclei [20]. The CCK2R-s are also expressed in vagal afferents.

Food intake enhances CCK production not only in the upper gastrointestinal tract but also in the hypothalamus [21, 22]. The peripheral administration of CCK induces satiety through CCK1R [23, 24] in rats and also in other animals [25], thus the „meal size“ will be smaller. Activation of central receptors (both CCK1R and CCK2R) may also decrease FI [15, 24, 26, 27].

Concerning the thermoregulatory actions, peripherally applied CCK in pharmacological doses elicited hypothermia [28] presumably by a vagal reflex causing hypometabolism, skin vasodilation and consequently increased HL. The effect is mediated through CCK1R.

Centrally injected CCK is known to induce fever-like coordinated changes in energy balance through CCK2R: an increase in MR, a decrease in HL, an elevation of Tc [29], and it also evokes anorexia [30]. Later it was shown that in endotoxin fever CCK2R-s are also involved [31].

1.3. 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 [32]. It is an alternative splice variant of the galanin-like peptide (GALP) mRNS. Alarin shows no affinity to the galanin receptors, but alarin receptors have not been discovered yet [33, 34]. Alarin has also been shown to be localized around the blood vessels with vasoactive actions [33] and may have a role in ocular blood flow regulation [35]. In addition, it increases the secretion of luteinizing hormone (LH) in male mice [36], has antidepressant-like [37-39] and antimicrobial [40] 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 arcuate nucleus (ARC), the dorsomedial (DMH), lateral and paraventricular (PVN) hypothalamic nuclei and the preoptic area [41]. 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. 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 [34, 36, 42, 43]. However, this orexigenic effect appears to be relatively weak compared to that of a major hypothalamic orexigenic mediator, neuropeptide Y (NPY) [34]. Effects of alarin on spontaneous nighttime FI or on fasting-induced re-feeding have not been fully investigated [34, 43].

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 [43].

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 [36, 43] or any change in oxygen consumption (indicating MR) in freely moving rats upon a central alarin injection [42].

2. AIMS

2.1 CCK in the regulation of energy balance

In the course of aging energy balance changes in two phases: first age-related obesity [44] appears followed by aging anorexia and weight loss [45].

Based on the earlier observed age-related shifts in the responsiveness of central catabolic melanocortin system [6, 8] and in that of its peripheral regulator, leptin [9, 10], 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 [11, 46-48], but to date, the effect of this peptide has not been investigated in middle-aged groups. Nutritional state has been shown to modify the age-related changes in the effects of the earlier investigated peptides [3, 10, 44, 49], 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.

2.2 Alarin in the regulation of energy balance

Although an acute orexigenic action of alarin has been described in rats and mice [34, 36, 42], 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 [36, 43] or any change in oxygen consumption in freely moving rats upon a central alarin injection [42]. 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 [50]) 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 intracerebroventricularly (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.

3. METHODS

3.1 Animals

Male rats from the colony of the Institute for Translational Medicine were used in the experiments. The lights were on between 06.00-18.00 h. The animals were kept at an ambient temperature of 22-25 °C. Standard rat chow (11 kJ/g) and tap water were continuously available (except the 24- or 48-h fasting periods). For the experiments on the effects of CCK, normally fed (NF) Wistar rats different age-groups have been established: 2, 4, 6, 12, 18 and 24 months old animals (NF2, NF4, NF6 and NF12, NF18 and NF24) represented juvenile, young adult, younger and older middle-aged, aging and old age-groups, respectively. 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-month-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 [42]. Such

animals have not finished the period of rapid growth and they may show altered FI-associated responses to regulatory peptides, therefore, we tested FI-related effects of alarin in 3 months old young adult as well as in juvenile, 6 weeks old Wistar and Long-Evans groups.

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.

Following experiments, the left retroperitoneal and epididymal fat pads were removed and weighed, along with the tibialis anterior muscle, as indicators of body composition [51]. All body composition indicators were calculated for 100 g body weight.

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.

3.2 Surgeries

Surgical interventions were performed under intraperitoneal (IP) ketamin + xylazine [78 mg/kg + 13 mg/kg] anesthesia. Animals received also 2 mg intramuscular gentamycin 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 [52]). 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.

3.3 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, in other cases at a pharmacological dose of 100 μg (88 nmol, as applied in earlier studies, [28] in a volume of 0.1 ml in metabolic studies, or by ICV injections at a dose of 500 ng (0.44 nmol) in a volume of 5 μl for both anorexigenic and metabolic tests.

To test dose dependence, alarin (GL Biochem) was administered at doses of 0.3, 1, 3 or 15 μg alarin (in a volume of 5 μl, ICV) at 9.00 h at various environmental temperatures. In other tests truncated alarin (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. 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, 2 mg/kg), a non-selective cyclooxygenase(COX)-inhibitor and meloxicam (Sigma, 1 or 2 mg/kg), a relatively selective COX-2 inhibitor were applied through an IP inserted polyethylene tube 30 min prior to ICV alarin injection.

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

3.4 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. In metabolic chambers of an Oxymax indirect calorimeter oxygen consumption (VO_2), respiratory quotient (RQ) and carbon-dioxide production (to determine MR) were measured by the help of an Oxymax gas analyzer and the data were electronically processed. During experiments the rats were semi-restrained in a cylindrical wire-mesh confiner. Together with this cylinder, they were placed into an open-circuit metabolic chamber, which in turn, was immersed into a thermostatically controlled water-bath to secure a standard 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 [53]), 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. Copper-constantan thermocouples were attached to the rats for measuring colonic (core) and tail skin temperatures (Tc and Ts, respectively): these – together with the cannula and 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 for electronic evaluation. Heat loss state ("heat loss index", HLI, as used in earlier studies; [54] was assessed from the relationship of the three monitored temperatures [HLI = (Ts-Ta) / (Tc-Ta)]. For administration the materials an injection cannula inserted into the chronically preimplanted ICV cannula was connected to

a 20-25-cm-long pp10 polythene tube [29]. 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.

3.5 Measurement of the food intake

For two weeks before the experiments rats were transferred into the automated Feed-Scale system to get habituated to the environment and to the powdered form of rat chow. This form of chow prevented food hoarding. 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.

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.

3.6 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 $p < 0.05$.

4. RESULTS AND DISCUSSION

4.1 Effects of CCK – a catabolic peptide

4.1.1. Results

Young adult NF4 rats responded with hypothermia to IP injection of a pharmacological dose of CCK, in line with data of the literature [28] due either to skin vasodilation (at a thermoneutral ambient temperature) or to decrease in metabolic rate (at a cool ambient temperature). In young adult NF4 rats the hyperthermic effects of ICV injected CCK appeared to be coordinated: immediate significant rise in oxygen consumption with skin vasoconstriction.

This metabolic response to ICV CCK was age-dependent. 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 non-significant in the NF18 and NF24 old age-groups. A negative linear correlation was shown between age and the CCK-induced change in Tc of individual rats.

In young adult rats the IP administered CCK caused significant suppression of 3-h cumulative FI during re-feeding after 48-h fasting. 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 (NF2) 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 1).

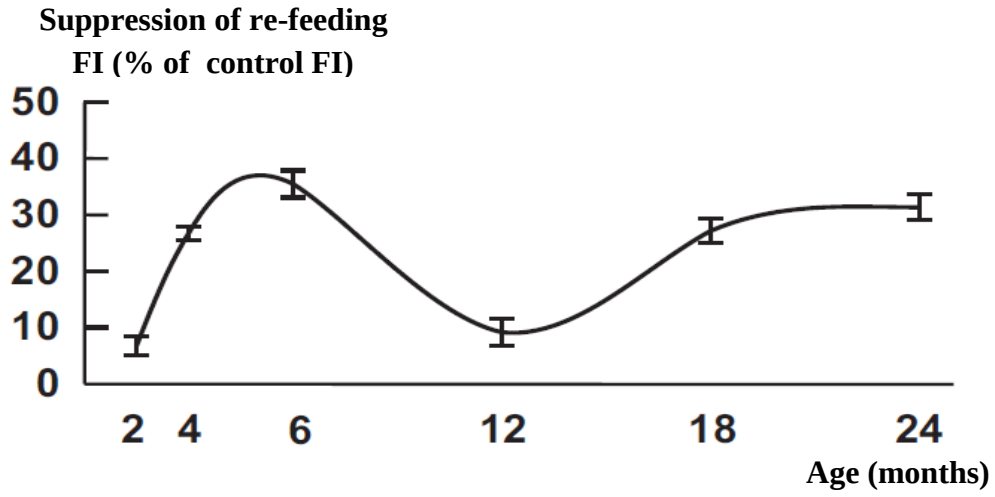

Figure 1. *Age-dependent changes in anorexigenic action of IP applied CCK*

Alterations in body composition influenced this pattern of CCK efficacy. Obese HF6 rats were resistant to the anorexic effect of CCK already at the age of 6 months, but the anorexic effect became again significant in HF12 animals. 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, unlike in NF12 animals.

The ICV injected CCK was also without significant effect on FI 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.

4.1.2. Discussion

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.

Both IP and ICV administrations of CCK 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 [6].

The anorexic effect of IP CCK observed in young adult rats is in line with data of the literature [55, 56]. 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 [15, 57, 58] to the NTS, brainstem, and further 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 [59].

Brain CCK2R-s might also be mediators of anorexia. Following food intake CCK is released in the hypothalamus [21], 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 [60]. Other studies demonstrated that CCK2R knockout mice are hyperphagic and obese [61] – their hypothalamic NPY expression was also high [62].

We hypothesized that after the young adult age either the peripheral or the central CCK effects may vary with further aging and possibly contribute to the alterations of energy balance during aging. Age-dependence has already been demonstrated for the effects of a number of peptides involved in the regulation of energy balance. 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 [6]. 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 [26] 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. Our 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 BW, 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 FI significantly. In addition, within the young adult group the dose of 1 μg was also able to induce similar and significant suppression of food intake in a similar setting as the 5 μg 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.

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 [11, 46, 47]. This results in suppressed level of hunger that is not altered very much by the relatively small postprandial CCK-release in old persons [47]. 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 [48].

Concerning our data, the above demonstrated changes in the efficacy to IP 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 [14] 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.

There are limited and controversial data concerning CCK production/effect 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 [63]. Nevertheless, various effects of exogenous CCK are not necessarily simultaneously enhanced [64, 65]. Other reports described suppression of gastrointestinal CCK gene- and protein expression [66] and reduced satiety in response to CCK [67, 68]. 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 [51, 69].

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. [26]. Considering that a decrease in metabolic rate is characteristic for old age [70], 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.

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 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 (dysorexia) [71].

Central CCK (CCK2R-s) may also participate in fever and sickness behavior [29, 72]. Aging is associated with diminished fever response [73].

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.

4.2 Effects of alarin – a catabolic peptide

4.2.1. Results

An acute ICV injection of 3 μg full-length alarin elicited a delayed significant increase in VO_2 at 25 ºC. This hypermetabolism and concurrent continuous tail skin vasoconstriction, 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.

Different doses of alarin (1, 3 or 15 μg) induced significant hyperthermia at 25 ºC, i.e. no dose-dependence was observed. The hyperthermic response was significant for each dose as compared with the effects of scrambled alarin. Scrambled alarin failed to induce any response. At cooler ambient temperatures the administration of 3 μg alarin resulted in similar Tc rises as those seen at 25 ºC (i.e. no hypothermia/hypometabolism).

Around the vasodilation threshold (28 ºC) a pronounced alarin-induced vasoconstriction was accompanied by a significant hyperthermic effect. Upon peripheral injections, full-length alarin failed to induce hyperthermia or vasoconstriction.

Truncated alarin, when administered together with ICV full-length alarin (3 μg), abolished the hyperthermic action of full-length alarin. Thus an antagonistic effect of alarin 6-25Cys was demonstrated.

Acute ICV injection of alarin (1, 3 or 15 μg) induced a significant increase in VO_2 as compared to vehicle treated animals at 25 ºC. 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. Even this high increase in Tc failed to elicit any compensatory vasodilation.

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. Peripheral pre-treatment of indomethacin (2 mg/kg, IP), a non-specific COX-inhibitor given 30 min before the ICV injection prevented alarin-induced hypermetabolism and hyperthermia. Both applied doses of meloxicam, a relatively selective COX-2 inhibitor, given 30 min prior to the ICV injection effectively reduced hypermetabolic and hyperthermic 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.

We tested 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. In contrast, NPY (5 μg, as positive orexigenic control) was able to induce FI in the same animal group. However, cumulative 24-h FI following alarin administration (at 3 μg, but not 1 μg dose) was found to be suppressed. Similarly, a central injection of NPY also reduced cumulative 24-h FI after its short-term orexigenic action. As these tests indicated a surprising anorexigenic action of alarin, we decided to test it in other settings.

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 in non-deprived rats.

Finally, alarin (3 μg) was given at 09:00 h to 24-h fasted rats at the onset of their 180-min re-feeding. The peptide suppressed re-feeding FI efficiently. In addition, this anorexigenic

effect also proved to be long-lasting, as shown by the significant suppression of 24-h FI of these animals.

Our results diverged from those of the literature. As those studies used younger (6 weeks old) Long-Evans rats [42], 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. NPY significantly enhanced FI both in Wistar rats and in Long-Evans rats.

4.2.2. Discussion

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 [36, 43, 74, 75].

Although a previous report failed to reveal any change in body temperature upon an ICV alarin injection in freely moving mice using biotelemetry [36], 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) [42], 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 [43], 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 [33], 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.

Alarin induces NPY release from hypothalamic explants similar to GALP, suggesting that alarin may exert its effect on feeding mediated by similar pathways [34]. However, no conclusive evidence (such as successful application of NPY antagonists to inhibit alarin effects) have been proposed for NPY mediation of alarin actions. 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 [76].

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 [77]. So far, GALP seems to elicit a fever-like hyperthermia [78], because it elicits hypermetabolism associated with a reduced heat loss [79]. 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 [77] 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 [80]. 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 [81]. 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 [42]. Although younger juvenile age groups of rats may exhibit different reactions compared to those of young adult (3 months old) animals [6], 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 [42] 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 [34].

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.

5. SUMMARY OF THE NOVEL FINDINGS

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.

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.

6. REFERENCES

1. Petervari, E., et al., *Alterations in the peptidergic regulation of energy balance in the course of aging.* Curr Protein Pept Sci, 2011. **12**(4): p. 316-24.
2. Kmiec, Z., et al., *Anorexia of aging.* Vitam Horm, 2013. **92**: p. 319-55.
3. Scarpace, P.J. and N. Tumer, *Peripheral and hypothalamic leptin resistance with age-related obesity.* Physiol Behav, 2001. **74**(4-5): p. 721-7.
4. Szekely, M., E. Petervari, and M. Balasko, *Thermoregulation, energy balance, regulatory peptides: recent developments.* Front Biosci (Schol Ed), 2010. **2**: p. 1009-46.
5. Szekely, M. and Z. Szelenyi, *Regulation of energy balance by peptides: a review.* Curr Protein Pept Sci, 2005. **6**(4): p. 327-353.
6. Petervari, E., et al., *Age-dependence of alpha-MSH-induced anorexia.* Neuropeptides, 2010. **44**(4): p. 315-22.
7. Rostas, I., et al., *Age-related alterations in the central thermoregulatory responsiveness to alpha-MSH.* J Therm Biol, 2015. **49-50**: p. 9-15.
8. Petervari, E., et al., *Central alpha-MSH infusion in rats: disparate anorexic vs. metabolic changes with aging.* Regul Pept, 2011. **166**(1-3): p. 105-11.
9. Rostas, I., et al., *Age-related changes in acute central leptin effects on energy balance are promoted by obesity.* Exp Gerontol, 2016. **85**: p. 118-127.
10. Petervari, E., et al., *Age versus nutritional state in the development of central leptin resistance.* Peptides, 2014. **56**: p. 59-67.
11. MacIntosh, C.G., et al., *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.* J Clin Endocrinol Metab, 2001. **86**(12): p. 5830-7.
12. Raybould, H.E., *Mechanisms of CCK signaling from gut to brain.* Curr Opin Pharmacol, 2007. **7**(6): p. 570-4.
13. Ondetti, M.A., et al., *Synthesis of cholecystokinin-pancreozymin. I. The C-terminal dodecapeptide.* J Am Chem Soc, 1970. **92**(1): p. 195-9.
14. Smith, G.P., C. Jerome, and R. Norgren, *Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats.* Am J Physiol, 1985. **249**(5 Pt 2): p. R638-41.
15. South, E.H. and R.C. Ritter, *Capsaicin application to central or peripheral vagal fibers attenuates CCK satiety.* Peptides, 1988. **9**(3): p. 601-12.
16. Gaudreau, P., et al., *Characterization and visualization of cholecystokinin receptors in rat brain using [3H]pentagastrin.* Peptides, 1983. **4**(5): p. 755-62.
17. Hill, D.R., et al., *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.* 1992.
18. Corp, E.S., et al., *Characterization of type A and type B CCK receptor binding sites in rat vagus nerve.* Brain Res, 1993. **623**(1): p. 161-6.
19. Cheng, C.A., et al., *Central and peripheral effects of CCK receptor antagonists on satiety in dogs.* Am J Physiol, 1993. **265**(2 Pt 1): p. G219-23.
20. Mercer, L.D., et al., *Direct visualization of cholecystokinin subtype2 receptors in rat central nervous system using anti-peptide antibodies.* Neurosci Lett, 2000. **293**(3): p. 167-70.
21. Schick, R.R., et al., *Sites in the brain at which cholecystokinin octapeptide (CCK-8) acts to suppress feeding in rats: a mapping study.* Neuropharmacology, 1990. **29**(2): p. 109-18.

22. Schick, R.R., et al., *Brain regions where cholecystokinin exerts its effect on satiety.* Ann N Y Acad Sci, 1994. **713**: p. 242-54.
23. Della-Fera, M.A. and C.A. Baile, *Cholecystokinin octapeptide: continuous picomole injections into the cerebral ventricles of sheep suppress feeding.* Science, 1979. **206**(4417): p. 471-3.
24. Shiraishi, T., *CCK as a central satiety factor: behavioral and electrophysiological evidence.* Physiol Behav, 1990. **48**(6): p. 879-85.
25. Moran, T.H. and K.P. Kinzig, *Gastrointestinal satiety signals II. Cholecystokinin.* Am J Physiol Gastrointest Liver Physiol, 2004. **286**(2): p. G183-8.
26. Akimoto, S. and K. Miyasaka, *Age-associated changes of appetite-regulating peptides.* Geriatr Gerontol Int, 2010. **10 Suppl 1**: p. S107-19.
27. Hirosue, Y., et al., *Cholecystokinin octapeptide analogues suppress food intake via central CCK-A receptors in mice.* Am J Physiol, 1993. **265**(3 Pt 2): p. R481-6.
28. Kapas, L., et al., *Cholecystokinin-octapeptide-induced hypothermia in rats: dose-effect and structure-effect relationships, effect of ambient temperature, pharmacological interactions and tolerance.* Neuropharmacology, 1987. **26**(2-3): p. 131-7.
29. Szelenyi, Z., et al., *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 Res, 1994. **638**(1-2): p. 69-77.
30. Gibbs, J., R.C. Young, and G.P. Smith, *Cholecystokinin decreases food intake in rats.* J Comp Physiol Psychol, 1973. **84**(3): p. 488-95.
31. Szekely, M., Z. Szelenyi, and M. Balasko, *Cholecystokinin participates in the mediation of fever.* Pflugers Arch, 1994. **428**(5-6): p. 671-3.
32. Santic, R., et al., *Gangliocytes in neuroblastic tumors express alarin, a novel peptide derived by differential splicing of the galanin-like peptide gene.* J Mol Neurosci, 2006. **29**(2): p. 145-52.
33. Santic, R., et al., *Alarin is a vasoactive peptide.* Proc Natl Acad Sci U S A, 2007. **104**(24): p. 10217-22.
34. Boughton, C.K., et al., *Alarin stimulates food intake and gonadotrophin release in male rats.* Br J Pharmacol, 2010. **161**(3): p. 601-13.
35. Schrodl, F., et al., *Distribution of the regulatory peptide alarin in the eye of various species.* Exp Eye Res, 2013. **106**: p. 74-81.
36. Fraley, G.S., et al., *Effects of alarin on food intake, body weight and luteinizing hormone secretion in male mice.* Neuropeptides, 2012. **46**(2): p. 99-104.
37. Wang, M., et al., *Antidepressant-like effects of alarin produced by activation of TrkB receptor signaling pathways in chronic stress mice.* Behav Brain Res, 2015. **280**: p. 128-40.
38. Zhuang, F., et al., *The antidepressant-like effect of alarin is related to TrkB-mTOR signaling and synaptic plasticity.* Behav Brain Res, 2016. **313**: p. 158-71.
39. Wang, M., et al., *Alarin-induced antidepressant-like effects and their relationship with hypothalamus-pituitary-adrenal axis activity and brain derived neurotrophic factor levels in mice.* Peptides, 2014. **56**: p. 163-72.
40. Wada, A., et al., *Alarin but not its alternative-splicing form, GALP (Galanin-like peptide) has antimicrobial activity.* Biochem Biophys Res Commun, 2013. **434**(2): p. 223-7.
41. Eberhard, N., et al., *Distribution of alarin immunoreactivity in the mouse brain.* J Mol Neurosci, 2012. **46**(1): p. 18-32.
42. Van Der Kolk, N., et al., *Alarin stimulates food intake in male rats and LH secretion in castrated male rats.* Neuropeptides, 2010. **44**(4): p. 333-40.

43. Fraley, G.S., et al., *Alarin 6-25Cys antagonizes alarin-specific effects on food intake and luteinizing hormone secretion.* Neuropeptides, 2013. **47**(1): p. 37-41.
44. Scarpace, P.J., M. Matheny, and E.W. Shek, *Impaired leptin signal transduction with age-related obesity.* Neuropharmacology, 2000. **39**(10): p. 1872-9.
45. Chapman, I.M., et al., *The anorexia of ageing.* Biogerontology, 2002. **3**(1-2): p. 67-71.
46. Di Francesco, V., et al., *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.* J Gerontol A Biol Sci Med Sci, 2005. **60**(12): p. 1581-5.
47. Serra-Prat, M., et al., *Effect of age and frailty on ghrelin and cholecystokinin responses to a meal test.* Am J Clin Nutr, 2009. **89**(5): p. 1410-7.
48. Ohta, M., et al., *Impaired release of cholecystokinin (CCK) from synaptosomes in old rats.* Neurosci Lett, 1995. **198**(3): p. 161-4.
49. Gabriely, I., et al., *Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process?* Diabetes, 2002. **51**(10): p. 2951-8.
50. Petervari, E., et al., *Thermoregulatory effects of alarin, Galanin meating, Neuroscience conference, San Diego.* 2013.
51. Soos, S., et al., *Anorexic vs. metabolic effects of central leptin infusion in rats of various ages and nutritional states.* J Mol Neurosci, 2010. **41**(1): p. 97-104.
52. Paxinos, G. and C. Watson, *The rat brain in stereotaxic coordinates, sixth ed. Academic, New York.* 2006.
53. Romanovsky, A.A., A.I. Ivanov, and Y.P. Shimansky, *Selected contribution: ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality.* J Appl Physiol (1985), 2002. **92**(6): p. 2667-79.
54. Romanovsky, A.A., A.I. Ivanov, and M. Szekely, *Neural route of pyrogen signaling to the brain.* Clin Infect Dis, 2000. **31 Suppl 5**: p. S162-7.
55. Smith, G. and J. Gibbs, *The satiating effect of cholecystokinin and bombesin-like peptides. G. Smith (Ed.).* 1998, Satiation: From Gut to Brain, Oxford University Press, New York, 97-125.
56. Balasko, M., et al., *Anorexic effect of peripheral cholecystokinin (CCK) varies with age and body composition (short communication).* Acta Physiol Hung, 2012. **99**(2): p. 166-72.
57. Blessing, W.W., *The Lower Brainstem and Bodily Homeostasis, (Ch. 7, Eating and metabolism) Oxford University,Press, New York, 323-372*. 1997.
58. Berthoud, H.-R., *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*. 2004.
59. Bi, S., et al., *Differential body weight and feeding responses to high-fat diets in rats and mice lacking cholecystokinin 1 receptors.* Am J Physiol Regul Integr Comp Physiol, 2007. **293**(1): p. R55-63.
60. Blevins, J.E., B.G. Stanley, and R.D. Reidelberger, *Brain regions where cholecystokinin suppresses feeding in rats.* Brain Res, 2000. **860**(1-2): p. 1-10.
61. Clerc, P., et al., *Involvement of cholecystokinin 2 receptor in food intake regulation: hyperphagia and increased fat deposition in cholecystokinin 2 receptor-deficient mice.* Endocrinology, 2007. **148**(3): p. 1039-49.
62. Chen, H., S. Kent, and M.J. Morris, *Is the CCK2 receptor essential for normal regulation of body weight and adiposity?* Eur J Neurosci, 2006. **24**(5): p. 1427-33.

63. Li, J., W. Ma, and S. Wang, *Slower gastric emptying in high-fat diet induced obese rats is associated with attenuated plasma ghrelin and elevated plasma leptin and cholecystokinin concentrations.* Regul Pept, 2011. **171**(1-3): p. 53-7.
64. Little, T.J., et al., *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.* Am J Physiol Regul Integr Comp Physiol, 2008. **294**(1): p. R45-51.
65. How, J.M., et al., *High-fat diet is associated with blunted splanchnic sympathoinhibitory responses to gastric leptin and cholecystokinin: implications for circulatory control.* Am J Physiol Heart Circ Physiol, 2011. **300**(3): p. H961-7.
66. Duca, F.A., et al., *Decreased intestinal nutrient response in diet-induced obese rats: role of gut peptides and nutrient receptors.* Int J Obes (Lond), 2013. **37**(3): p. 375-81.
67. Covasa, M. and R.C. Ritter, *Rats maintained on high-fat diets exhibit reduced satiety in response to CCK and bombesin.* Peptides, 1998. **19**(8): p. 1407-15.
68. Torregrossa, A.M. and G.P. Smith, *Two effects of high-fat diets on the satiating potency of cholecystokinin-8.* Physiol Behav, 2003. **78**(1): p. 19-25.
69. Soos, S., et al., *Complex catabolic effects of central alpha-MSH infusion in rats of altered nutritional states: differences from leptin.* J Mol Neurosci, 2011. **43**(2): p. 209-16.
70. McGandy, R.B., et al., *Nutrient intakes and energy expenditure in men of different ages.* J Gerontol, 1966. **21**(4): p. 581-7.
71. Roberts, S.B., et al., *Control of food intake in older men.* JAMA, 1994. **272**(20): p. 1601-6.
72. Weiland, T.J., N.J. Voudouris, and S. Kent, *CCK(2) receptor nullification attenuates lipopolysaccharide-induced sickness behavior.* Am J Physiol Regul Integr Comp Physiol, 2007. **292**(1): p. R112-23.
73. Buchanan, J.B., E. Peloso, and E. Satinoff, *Thermoregulatory and metabolic changes during fever in young and old rats.* Am J Physiol Regul Integr Comp Physiol, 2003. **285**(5): p. R1165-9.
74. Eberhard, N., et al., *Distribution of alarin immunoreactivity in the mouse brain.* Journal of Molecular Neuroscience, 2012. **46**(1): p. 18-32.
75. Webling, K.E., et al., *Galanin receptors and ligands.* Front Endocrinol (Lausanne), 2012. **3**: p. 146.
76. Szekely, M., et al., *Acute, subacute and chronic effects of central neuropeptide Y on energy balance in rats.* Neuropeptides, 2005. **39**(2): p. 103-15.
77. Lawrence, C.B., F.M. Baudoin, and S.M. Luckman, *Centrally administered galanin-like peptide modifies food intake in the rat: a comparison with galanin.* J Neuroendocrinol, 2002. **14**(11): p. 853-60.
78. Szekely, M. and Z. Szelenyi, *Endotoxin fever in the rat.* Acta Physiol Acad Sci Hung, 1979. **53**(3): p. 265-77.
79. Kageyama, H., et al., *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, 2013. **50**(3): p. 443-52.
80. Harvey, C.A. and A.S. Milton, *Proceedings: Endogenous pyrogen fever, prostaglandin release and prostaglandin synthetase inhibitors.* J Physiol, 1975. **250**(1): p. 18P-20P.
81. Johnson, R.W., *The concept of sickness behavior: a brief chronological account of four key discoveries.* Vet Immunol Immunopathol, 2002. **87**(3-4): p. 443-50.

7. 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. Pétervári Erika 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. Márta Balaskó, prof. Miklós Székely and Nóra Füredi. 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-Mihálffy, Adrienn Bóka-Kissné and Margit Koncsecskó-Gáspár 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.

PUBLICATIONS, CITABLE ABSTRACTS

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 (2013)
2. 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