

CENTRAL METABOLIC EFFECTS OF THE CORTICOTROPIN SYSTEM IN THE COURSE OF AGING

Doctoral (Ph.D.) Thesis

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1. INTRODUCTION

During the course of aging, long-term trends emerge in the regulation of energy balance resulting in middle-aged obesity and aging anorexia that in turn leads to cachexia and also to sarcopenia of old age [Morley 2001], [Di Francesco et al. 2007], [Pétervári et al. 2011a]. Both energy excess and deficit present important worldwide challenges affecting more and more people with serious impact on health status and health cost. That makes it essential to achieve deeper understanding regarding the functions and mechanisms of healthy and pathological energy processes and it underlines the importance of research aimed at age-related alterations in energy metabolism.

1.1. Peptidergic regulation of the energy homeostasis

Regulation of body weight (BW) may be considered to be a homeostatic system. The central nervous system receives various forms of afferent signals from the periphery, which modify the activity of central neurotransmitters, modulator substances, and consequently, efferent mechanisms are activated. Some afferent signals represent the actual feeding state, while others, called adiposity signals indicate the nutritional state [Wilding 2002], [Székely and Szélényi 2005]. One of the most important adiposity signals is leptin, which (among other sites) binds primarily to receptors of the nucleus arcuatus of the hypothalamus (ARC) [Palkovits 2003], [Székely and Szélényi 2005]. An elevated level of leptin stimulates the expression of anorexigenic neuropeptides [*i.e.* those inhibiting food intake (FI)] in the ARC, such as alpha- melanocyte- stimulating hormone (alpha- MSH) and its precursor pro-opiomelanocortin [Palkovits 2003]. Alpha- MSH activates ‘second-order’ neurons in the paraventricular nucleus of the hypothalamus (PVN), that are responsible for the secretion of further catabolic peptides (*i.e.* anorexigenic and hypermetabolic), like corticotropin-releasing factor (CRF) [Valassi et al. 2008].

Earlier studies demonstrated age-related shifts in the responsiveness to centrally administered catabolic mediators such as leptin [Pétervári et al. 2014] or alpha- MSH [Pétervári et al. 2010], [Rostás et al. 2015]. Intracerebroventricular (ICV) leptin injection- and infusion-induced anorexia was strong in the youngest groups, weaker in aging rats and became more pronounced again in the oldest group. In contrast, leptin-induced hypermetabolism declined continuously with aging [Pétervári et al. 2014], [Rostás et al. 2016]. Central acute and chronic anorexigenic effects of alpha-MSH showed similar patterns during aging to those of leptin administration [Pétervári et al. 2010], [Pétervári et al. 2011b]. A similar age-related pattern was observed also in the hypermetabolic effects of central alpha-MSH injections [Rostás et al. 2015], while infusions of this peptide induced weak hypermetabolic response in the youngest group and strong hypermetabolic effects in older animals [Pétervári et al. 2011b]. Based on these observations, the question arises, whether CRF, which lays downstream to leptin and melanocortins may also contribute to age-associated obesity and/or to the development of aging anorexia and sarcopenia.

1.2. Corticotropin- releasing factor (CRF)

CRF is a 41-aa peptide, which is produced predominantly in the PVN [Morin et al. 1999], but it has been also detected in the cerebral cortex, locus coeruleus, medial preoptic area, stria terminalis, amygdala or in the hippocampus [Morin et al. 1999], [Janssen and Kozicz 2013]. In the PVN CRF level is increased in states of energy excess or poor glucose utilization [Seeley et al. 1996], whereas, states of negative energy balance like fasting or cold exposure reduce CRF and CRF receptor level [Fekete et al. 2000]. Age may also have an impact on the amount of CRF in the central nervous system. Several studies have reported increased hypothalamic CRF expression in elderly humans and old rats, but others detected unchanged hypothalamic CRF expression [Bao and Swaab 2007], [Aguilera 2011].

1.2.1. Effects of CRF

There is a considerable body of evidence indicating that CRF and other members of the corticotropin family are endogenous catabolic agents, their anorexigenic and hypermetabolic effects result in weight loss [Richard et al. 2002]. During acute stress CRF reduces FI through the stimulation of anorexigenic neurons in the ARC [Chrousos 2000], although CRF is able to decrease FI even in the absence of stress [Crespi et al. 2004]. Acute central administration of CRF suppresses spontaneous food consumption and fasting-induced re-feeding [Morley, 1987], [Cullen et al. 2001], which were accompanied by hypermetabolism and increased brown-fat thermogenesis [LeFeuvre et al. 1987], [Carlin et al. 2006]. Not only acute, but also chronic central administration of CRF has catabolic effects, it enhanced the weight of brown adipose tissue [Cullen et al. 2001] and caused a fall in BW partly by suppression of FI partly by enhancement of metabolism [Arase et al. 1988]. Thus, both acute and chronic central administration of CRF evokes catabolic effects, that appear to be coordinated.

In addition to its roles in energy homeostasis CRF, as a member of the hypothalamo-pituitary- adrenal (HPA) axis, participates in stress processes and in the development of anxiety and depressive disorders [Bale and Vale 2004], [Fekete and Zorilla 2007], [Janssen and Kozicz 2013]. It has been proven, that CRF plays an important role in thermoregulation, both central CRF injection and infusion were shown to induce hyperthermia [Buwalda et al. 1997], [Heinrichs et al. 2001], [Richard et al. 2002]. In addition, this peptide is known to influence the motor activity [Contarino et al. 2000], [Ohata and Shibasaki 2004], the immune- [De Souza 1995] and the reproductive systems [Heinrichs and Richard 1999], reward- [Koob 2013] and learning mechanisms [Hashimoto et al. 2001].

1.2.2. Receptors of the corticotropin system

Two subtypes of G-protein coupled receptors (CRF1 and CRF2) mediate the effects of corticotropins. CRF elicits its effects by binding predominantly to CRF1 and to a lesser extent to CRF2 receptors [Perrin and Vale 1999], [Reul and Holsboer 2002].

CRF1 receptors are found mainly in hypothalamic nuclei, in the anterior pituitary, in the amygdala and in the cerebral cortex [Van Pett et al. 2000], [Reul and Holsboer 2002]. Anxiogenic actions, depressive behavior, increased locomotor activity and

hyperthermic/hypermetabolic effects have been attributed to the activation of this receptor type [Van Pett et al. 2000], [Reul and Holsboer 2002], [Figueiredo et al. 2010]. It is also capable to evoke moderate anorexigenic effects in emotional stress [Hotta et al. 1999].

CRF2 receptor expression appears to be more restricted in the brain relative to that of CRF1 receptors. Its main expression sites are the ventromedial nucleus in the hypothalamus and the lateral septum [Van Pett et al. 2000], [Fekete and Zorilla 2007]. Various studies established the primary role of CRF2 receptor in mediating the anorexigenic actions of CRF [Cullen et al. 2001], [Stengel and Taché 2014]. Moreover, CRF2 receptors are known to mediate the late phase of the stress response and anxiolytic-, antidepressive behavior [Van Pett et al. 2000], [Reul and Holsboer 2002].

2. PRIMARY HYPOTHESES AND AIMS

I. According to our first hypothesis acute catabolic (*i.e.* anorexigenic and hypermetabolic) CRF effects contribute to middle-aged obesity and aging anorexia. We also hypothesized, that catabolic CRF effects vary with aging similarly to those of melanocortins and leptin. Moreover, acute catabolic effects of CRF change in differential, non-parallel, *i.e.* disparate ways in male and female Wistar rats during the course of aging.

Therefore we aimed

1. to assess anorexigenic and hypermetabolic effects of ICV CRF injection in different age-groups of male and female Wistar rats,
2. to analyse the potential involvement of acute central catabolic CRF effects in the development of age-related obesity and aging anorexia.

II. We also hypothesized, that age-related changes in central chronic CRF effects take part in the development of the special pattern of long-term BW regulation in male rats, similar to the catabolic activators of CRF.

Thus, we aimed

1. to investigate the anorexigenic and hypermetabolic responsiveness to a 7-day ICV CRF infusion in different age-groups of male Wistar rats,
2. to analyse whether age-related variations of CRF effects may also contribute to middle-aged obesity and aging anorexia leading to weight loss in old age-groups.

III. We proposed that age-related changes of endogenous CRF activity in the PVN contribute to the life-long BW development in male rats.

Thus, we aimed to analyze mRNA expression of CRF in the PVN in different age-groups of male Wistar rats. We also aimed to assess the potential contribution of the CRF expression to the above mentioned long-term BW development.

3. MATERIALS AND METHODS

3.1. Animals

Various age- groups of male and female Wistar rats were used in the experiments of the present studies from the Colony of the Institute for Translational Medicine of the Medical School, University of Pécs, Hungary. In acute experiments ICV CRF or pyrogen- free saline (PFS) injections were administered to young adult (3- months old), younger and older middle-aged (6- and 12- months old), aging and old (18- and 24- months old) male and female rats. For thermoregulatory analysis and for tests of FI separate groups of animals had to be used. However, in chronic experiments 7-day ICV CRF- or PFS infusions were applied to 3-, 12-, 18- and 24-months old male rats. For CRF gene expression analysis different age- groups (aged 3-, 12-, 18- or 24- month) of intact male rats were used. Following experiments, animals were sacrificed; no repeated testing across age-groups was possible.

After they have reached the appropriate age, rats were housed in individual plastic home-cages under controlled illumination conditions (with 12:12 hours dark-light regime, lights were on from 06:00 am) and at an ambient temperature of 24-25 °C. Standard rat chow (11 kJ/g; CRLT/N rodent chow, Szindbád Kft., Gödöllő, Hungary) and tap water were available *ad libitum*, except for the 24-h fasting period in acute experiments when only water was provided for the appropriate groups. Spontaneous daily FI and BW were measured every day at 09.00 h, consequently the animals were habituated to regular handling.

All experimental interventions and procedures were in good accord with institutional (University of Pécs, Medical School) and international standards (86/609/EEC, Directive 2010/63/EU of the European Parliament and of the Council).

3.2. Substances applied

During our experiments corticotropin-releasing factor (CRF-41, Bachem, AG Switzerland) dissolved in PFS or PFS alone (as control) was delivered into the right lateral cerebral ventricle.

In acute experiments 5 µl CRF solution or PFS was applied. Each animal in each age-group received CRF and its solvent in random order. Based on previous reports of the literature [Zorilla et al. 2004], [Semjonous et al. 2009] and our observations, two different doses of CRF injection were used for tests of FI after 24-h fasting (0.3 µg CRF) and for thermoregulatory analysis (1 µg CRF).

In chronic tests CRF solution or PFS alone was administered via Alzet osmotic minipumps at a flow rate of 1 µl/h for 7 days. The applied dose of CRF (0.2 µg/µl/h) was chosen according to earlier observations [Rivest et al. 1989].

3.3. Surgeries

In acute experiments after an at least 7-day adaptation to the experimental systems and at least 7 days before the ICV injection, an ICV guide cannula was implanted into the right lateral ventricle using a stereotaxic apparatus (coordinates relative to bregma: A: -1.0 mm, L: 1.5 mm, V: 3.5 mm) according to the Rat Brain Atlas [Paxinos and Watson 2006]. Several

days before the first experiment, angiotensin II (Sigma, A9525, 20 ng/5 μ l) was injected through the implanted cannula in order to check its correct location. Appropriate location was assumed if at least 5 ml water was consumed within 30 min.

In chronic experiments after at least one week of adaptation to the biotelemetric system and 5-7 days prior to the start of the infusion, an e-mitter (HR E-Mitter, Sunriver, OR) was implanted intraperitoneally into the rats. The ECG electrodes of the e-mitter were attached to the chest of the animals subcutaneously. After full recovery from the e-mitter surgery a Brain-Infusion-Kit (Alzet) was implanted intracerebroventricularly with the same coordinates as those of the ICV cannula in acute experiments. Simultaneously an Alzet osmotic minipump filled with the appropriate solution was inserted subcutaneously underneath the nape of the neck and was attached to the Brain-Kit.

All surgical interventions were performed under intraperitoneal (IP) ketamine + xylazine [78 mg/kg (Calypsol, Richter) + 13 mg/kg (Sedaxylan, Eurovet)] anesthesia. Moreover Gentamycin injection (2 mg IP) was also applied to avoid infections.

3.4. Experimental methods

3.4.1. Assessment of central acute CRF effects on food intake

To assess the anorexigenic effects of the CRF injection, an automated FeedScale system (Columbus, OH) was used. The system allowed continuous recording of the amount of consumed food and prevented food hoarding. Data were registered every 10 minutes. On day 1 at 09.00 h food was removed for 24-h. Five minutes before the re-feeding started (on day 2 at 09.00 h) assigned rat groups received ICV CRF or PFS injection to test the inhibitory effect of the peptide on 3-h cumulative FI.

3.4.2. Assessment of central acute CRF effects on thermoregulation and metabolic rate

During acute experiments thermoregulatory analyses were performed in an indirect calorimeter (Oxymax, Equal Flow, Columbus, OH). The tests were performed between 09.00 h and 15.00 h and data was registered in 10-min intervals for 3 hours. Semi-restrained rats were singly enclosed in metabolic chambers immersed into a thermostatically controlled water-bath. Oxygen consumption [VO_2 , representing metabolic rate] was determined by indirect calorimeter from the air flowing through the chambers. For the recording of core temperature (T_c) in the colon, tail skin- (T_s) and ambient temperatures (T_a), copper-constantan thermocouples were applied and data were collected by a Digi-Sense Benchtop Thermometer (Cole-Parmer). The rate of heat loss (heat loss index, HLI) was calculated from the relationship of the monitored temperatures: $\text{HLI} = (T_s - T_a) / (T_c - T_a)$ [Romanovsky and Blatteis 1996]. HLI near 0 suggests vasoconstriction as a heat conserving mechanism, HLI near 1 shows vasodilation as indication of heat loss.

3.4.3. Biotelemetric measurements during central chronic CRF administration

During chronic experiments, a biotelemetric system (MiniMitter-VMFH series 4000, Sunriver, OR) was applied. The intraperitoneally implanted e-mitter (with subcutaneous ECG electrodes) detected Tc, heart rate (HR, representing metabolic rate) and spontaneous horizontal locomotor activity (Act). The system registered data every 5 minutes that were integrated into mean values of 12-h periods, equivalent to the daytime inactive and to the nighttime active phases. For primary data analysis, the VitalView software provided by the manufacturer (MiniMitter) was used. In this biotelemetric system FI and BW were measured manually daily.

3.4.4. CRF gene expression analysis in the paraventricular nucleus using quantitative real-time polymerase chain reaction (qRT-PCR)

CRF gene expression analysis was performed in collaboration with the Department of Anatomy and Department of Pharmaceutical Biotechnology of University of Pécs. The RNA was isolated with the Pure Link™ RNA Mini Kit (Life Sciences, Carlsbad CA, USA) from PVN samples of different age-groups of intact male Wistar rats as described previously [Füredi et al. 2016]. The total amount of RNA was determined by NanoDrop (Thermo Scientific). High-capacity cDNA kit was applied (Applied Biosystems, Foster City, CA, USA) to perform cDNA synthesis and for CRF gene expression analysis, qRT-PCR was performed using SensiFast SYBR Green reagent (BioLine). Amplifications were run on ABI StepOnePlus system, its software was used to analyze gene expression, which was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene. PCR conditions were set according to previous studies [Füredi et al. 2016] and the amplification of PCR products were calculated according to the $2^{-\Delta\Delta Ct}$ method.

3.4.5. Other *post mortem* examinations

After acute and chronic experiments rats were euthanized and the position of the injection sites were checked macroscopically using brain coronal sections of the removed and fixed brains. Only rats with appropriate cannula location were included in the analysis. Indicators of body composition of the animals (epididymal and retroperitoneal fat, anterior tibial muscle) that received ICV infusion were removed and weighed.

3.5. Statistical analysis

Each animal group contained at least 6 rats. SPSS 11.0 for Windows was used for the statistical analysis of the data with application of one-way, two-way (univariate or repeated-measures analysis) or repeated-measures ANOVA complemented by Tukey's *post hoc* test, when more than two groups were compared. The significance was set at the level of $p < 0.05$.

4. RESULTS

4.1. Acute anorexigenic CRF effects in male and female rats during the course of aging

In young adult and younger and older middle-aged male rats the ICV administered CRF injection caused a strong suppression of 3-h cumulative FI during re-feeding following 24-h fasting. The peptide failed to induce a significant anorexigenic response in aging and old rats. These results suggest that the anorexigenic effects of an acute central injection of CRF show a gradual decline with aging in males.

In female rats, the most pronounced anorexigenic effects were detected in young adults, although they were also significant in the older age-groups. Therefore, acute central CRF administration- induced anorexigenic effects were maintained at a significant level in all female age-groups.

4.2. Acute hypermetabolic/hyperthermic CRF effects in male and female rats during the course of aging

The young adult male age-group showed a CRF-induced increase in VO_2 , with a consequent significant rise in T_c . No compensatory activation of heat loss was detectable, as indicated by HLI. Based on these data, CRF-induced hyperthermia appears to be coordinated, in which case both heat production and heat conservation (lack of vasodilation) promote the rise in T_c .

Regarding age-related variations of these reactions, similar CRF-induced hyperthermic and hypermetabolic reactions were observed (regarding maximal T_c and VO_2 changes) of young and older male rats. Although these responses proved to be significant in all age-groups, the maximal increase in T_c and VO_2 declined with aging. Heat loss mechanisms did not show any age-related alteration.

Young adult and younger middle-aged female rats showed a significant CRF-induced increase in metabolic rate and T_c without vasodilation, indicated by HLI. However, this response was weaker than in the corresponding male groups. On the other hand older female age-groups failed to show any significant change in T_c or VO_2 . Thus, the hyperthermic/hypermetabolic effects of CRF showed a gradual age-dependent decline in female rats.

4.3. Effects of chronic CRF administration on body weight and body composition in male rats during the course of aging

Body composition values and mean BW-s of different age-groups of control animals were in accord with those observed in our previous studies [Pétevári et al. 2010], [Balaskó et al. 2013]: BW of young adult rats was significantly lower than that of older animals. Concerning body composition indicators, BW of young adult rats was significantly lower than that of older animals. Concerning body composition indicators, epididymal fat values were found to be significantly higher in 12- or 18-month groups than in the youngest and the oldest observed animals, whereas retroperitoneal fat pad of young adult rats differed significantly

from values of 18-month, aging animals. No difference in muscle mass was detected in any group, except for the oldest sarcopenic animals (24- vs. 12 months of age).

Regarding the age-related effects of a 7-day ICV CRF infusion on BW values, CRF treatment suppressed BW throughout the infusion period in the 3-month, 18- and 24-month age-groups, but not in middle-aged animals. By the end of the CRF infusion, significant reduction of retroperitoneal fat developed in the oldest groups, while no change occurred in epididymal fat or muscle mass in any group.

4.4. Chronic anorexigenic CRF effects in male rats during the course of aging

Concerning the anorexigenic effects, ICV CRF infusion elicited the strongest suppression during the first two days in all rats. Significant anorexia was detected for 2 days in the 3-month, for 7 days in the 18- and 24-month animals, whereas no change was observed in middle-aged animals.

Measurements of 7-day cumulative energy intake in CRF-treated vs. respective control groups demonstrated the age-dependence and short-term feature of CRF-induced anorexia. Anorexigenic effects of the CRF infusion were strongest in the oldest rats.

4.5. Results of biotelemetric measurements in chronic CRF administration in male rats during the course of aging

Our data demonstrate that mean nighttime control HR values of young adult rats differed from those of older age-groups. These results are in accord with our previous findings that demonstrated a decline in the control nighttime HR values of rats in the course of aging [Pétervári et al. 2014]. During the ICV CRF infusion mean daytime HR (inactive period, nadir of the circadian rhythm) failed to show significant increase. A slight rise in HR was detected in middle-aged and old rats on day 1 of the infusion, which is likely to be attributable to the surgery.

Mean basal (pre-infusion) day- and nighttime body temperature values did not change across our age-groups. Regarding hyperthermic effects of CRF, the infusion induced a 2-day elevation of mean daytime temperatures in young adult rats. Otherwise only a slight rise was detected in the mean daytime T_c value of day 1 in aging animals. This rise was probably due to the surgery, while differences in other groups did not reach statistical significance.

Act of young adult rats exhibited some diminishment on day 2 of the infusion that did not reach statistical significance. Otherwise no CRF-related alteration of Act was detectable.

4.6. CRF gene expression in the paraventricular nucleus in male rats during the course of aging

In our study qRT-PCR measurements revealed that in the PVN of different age-groups of male rats, CRF mRNA expression showed significant changes with aging. CRF mRNA expression appeared to increase until 18 months of age with a subsequent slight decline in the 24-month animals. *Post hoc* analysis showed a significant difference between the young and aging groups. In addition, a rising tendency was observed between the middle-aged and aging groups.

5. DISCUSSION OF FINDINGS

5.1. Age- and gender- dependence of central acute anorexigenic CRF effects

In males, acute ICV CRF administration-induced suppression of re-feeding was pronounced in the young adult and also in the younger and older middle-aged groups, whereas this effect failed to develop in older animals. This age-related pattern distinctly differs from that of acute central alpha-MSH- and also from that of leptin- induced FI suppression [Pétervári et al. 2010] [Rostás et al. 2016]. The age-related patterns characterizing alpha-MSH- and leptin-induced anorexia potentially contributes to the explanation of middle-aged obesity and aging anorexia. In contrast, the age-related pattern derived from our experiments, characterizing acute central CRF-anorexia is unlikely to promote the development of either the middle-aged obesity or the aging anorexia in male Wistar rats.

CRF2 receptors may play a decisive role in the above described age-related pattern, since the vast majority of the related literature attributes anorexigenic CRF effects to CRF2 receptor mediation [Cullen et al. 2001], [Ohata and Shibasaki 2004], [Stengel and Taché 2014]. In addition, contribution of CRF1 receptors to anorexigenic effects is mainly attributed to emotional stress [Hotta et al. 1999] and their activation results in the increase in peripheral plasma corticosterone level, which has been shown to be coupled with increased FI [Chrousos 2000]. Thus, our present findings may have implications for the potential lack of involvement of CRF2 receptors in aging-induced variations of BW in male rats.

In females, CRF-induced anorexia was maintained in all age-groups. This continuous anorexigenic efficacy of CRF may have helped to prevent rapid weight gain in middle-aged and older female animals, but apparently failed to induce weight loss up to 24 months of age. However, it cannot be excluded that even older age-groups of female rats (*e.g.* 30-month) would show anorexia and weight loss as a result of this maintained efficacy. In summary, a gender difference emerges in the age-related patterns of CRF-induced anorexia and of CRF2 receptor responsiveness in Wistar rats: these CRF effects decline in males, but they are maintained in females during aging (until 24 months of age).

5.2. Age and gender-dependence of central acute hyperthermic/ hypermetabolic CRF effects

Concerning hypermetabolic/hyperthermic effects in young adult males, ICV CRF administration elicited a prompt rise in VO_2 and induced a steady rise in T_c , which was accompanied by continuous heat conservation as indicated by the HLI. This thermoregulatory response appears to be similar to fever-like coordinated hyperthermias [Balaskó et al. 2013]. Some studies suggested a role of CRF in the development of fever in a prostaglandin-independent way [Figueiredo et al. 2010], while others consider prostaglandins to be important factors in it [Telegdy and Adamik 2008]. However, antipyretic effects of centrally applied CRF were also demonstrated previously [Holdeman et al. 1985]. Our results failed to detect compensatory vasodilation arguing for a coordinated hyperthermic response.

Regarding the age-related pattern of hypermetabolic/hyperthermic acute central CRF actions in males, these effects were significant across all age-groups, although a decline began in the young middle-aged group. This pattern was similar to those of leptin injection in male

Wistar rats [Rostás et al. 2016] and may contribute to the development of middle-aged obesity. These findings may also suggest the potential involvement of CRF1 receptors in aging-induced variations of BW, since hyperthermic CRF effects were shown to be mediated predominantly by CRF1 receptors [Figueiredo et al. 2010].

Young female rats exhibited a much weaker hypermetabolic/hyperthermic response upon acute central CRF administration as compared with that of males. This phenomenon cannot be explained by a diminished thermogenic capacity of females, as febrile responses of male and female rats to toxic agents did not differ in previous studies [Gordon and Mack 2003]. Moreover, heat production capacity of the brown adipose tissue was even enhanced in female rats [Justo et al. 2005].

5.3. Age-dependence of central chronic CRF effects on body weight and body composition

During the course of the ICV CRF infusion young adult rats showed significant, but weaker BW reduction compared with the oldest animals (18-, 24-month), which showed strong weight loss, while middle-aged male rats failed to lose weight. This pattern was similar to those of alpha-MSH and leptin infusions [Pétervári et al. 2011b], [Pétervári et al. 2014] and it suggests the potential contribution of CRF in age-related body weight changes.

Regarding body composition indicators ICV CRF infusion induced the biggest change in the two oldest age-groups. Aging and old rats showed a loss of retroperitoneal fat tissue by the end of the 7-day infusion, whereas epididymal fat did not show any decline. Although CRF-infusion-induced decreases in fat mass were reported previously by other researchers in young age-groups of different rat strains [Arase et al. 1988], [Cullen et al. 2001], in the present study no change in fat mass indicators was detected either in young adult, or in middle-aged groups. In previous experiments chronic CRF1 receptor activation was shown to increase fat mass by the end of the infusion [Cullen et al. 2001], thus failure of fat accumulation in rats of our study supports the dominance of CRF2 receptor activation.

Muscle mass indicators did not show any CRF-induced change in any group, indicating a lack of sarcopenic effects of our 7-day CRF infusion. This lack of sarcopenia (a typical consequence of overactivity of glucocorticoids) [Kayali et al. 1987] in all our CRF-treated age-groups also indicates that effects of CRF-induced HPA axis activation [Rivest et al. 1989], [Cullen et al. 2001] and those of the inevitable rise in peripheral corticosterone level [Rivest et al. 1989], [Cullen et al. 2001] did not influence our results significantly. On the other hand, enhancement of CRF-induced weight loss in the oldest age-groups in our study may be, at least in part, ascribed to the relative diminishment of peripheral glucocorticoid release in old rats, as demonstrated by previous studies [Rebuffat et al. 1992].

5.4. Age-dependence of central chronic anorexigenic CRF effects

With regard to CRF infusion-induced anorexia, the FI suppression was of short duration in the young, but strong and persistent throughout the infusion in the two oldest age-groups. The above described findings strongly support the contribution of CRF in aging anorexia and cachexia and they do not contradict a potential role of these changes in middle-aged obesity. This age-related pattern was similar to those of activators of CRF such as

melanocortin agonist alpha- MSH or adipose tissue derived leptin [Péteřváři et al. 2011a], [Péteřváři et al. 2014].

As CRF also activates the HPA axis, the question arises, as to what extent would the inevitable rise in peripheral corticosterone level [Rivest et al. 1989], [Cullen et al. 2001] contribute to the CRF infusion-induced changes in energy balance. Previous reports demonstrated that a 13-day ICV CRF-infusion increased the serum level of corticosterone [Rivest et al. 1989], [Cullen et al. 2001]. However, central injections of CRF proved to be efficient in suppressing FI even in hypophysectomized rats [Morley and Levine 1982]. This finding argues against a crucial role of peripheral corticosterone in CRF effects. Moreover corticosteron is reported to increase FI by inhibition of CRF and stimulation of orexigenic peptides [Chrousos 2000]. Thus, age-related diminishment of glucocorticoid release (antagonizing central anorexigenic CRF effects) from the adrenal cortex of old rats upon ACTH activation [Rebuffat et al. 1992] may take part in the enhanced anorexigenic effects of CRF in the older age-groups.

Potential contribution of other factors, such as divergent age-related alterations in the activity of different subpopulations of CRF receptors may be also considered. In the present study, an important role of the CRF2 receptor emerges, since several studies have demonstrated the CRF induces anorexia primarily via CRF2 receptors [Cullen et al. 2001], [Ohata and Shibasaki 2004], [Stengel and Taché 2014].

5.5. Age- dependence of central chronic CRF effects on metabolic rate and locomotor activity

A transient moderate CRF-induced hyperthermia was observed in the young adult group, but no change of Tc (day- or nighttime) developed in middle-aged or older rats. In addition, increase of HR failed to reach a significant extent in any age-group.

Hyperthermia/hypermetabolism induced by an ICV CRF infusion differed from those induced by the infusions of leptin or alpha- MSH [Péteřváři et al. 2011b], [Péteřváři et al. 2014]. Hypermetabolic effects of the ICV CRF infusion, at a dose appropriate for induction of anorexia in young adult rats, appear to be much weaker than those of corresponding doses of melanocortins or leptin [Péteřváři et al. 2011a], [Péteřváři et al. 2014] and did not show any remarkable age-related variations.

The lack of increase in HR during the CRF infusion supports the potential contribution of CRF2R activation to the observed effects. Previous studies reported acute elevations of HR upon acute central CRF injections based on the activation of CRF1 receptor [Nijsen et al. 2000], whereas specific agonist of CRF2 receptor is able to elicit bradycardia [Nakamura et al. 2009]. Here again, we hypothesize the mutual quenching of effects of CRF1 and CRF2 receptors concerning HR.

Apart from some surgical procedure-induced suppression Act failed to change in CRF-, alpha- MSH- or leptin-treated rats alike in all age-groups [Péteřváři et al. 2011a], [Péteřváři et al. 2014]. Previous studies reported activity-enhancing effects of CRF1 receptor [Contarino et al. 2000], and inhibitory influence of CRF2 receptor on motor activity [Ohata and Shibasaki 2004]. So, the lack of rise in nighttime Act may indicate CRF infusion-induced

additional activation of CRF2 receptors, counteracting the locomotor activity–inducing effects of the inevitable CRF1 receptor activation.

5.6. Age-dependence of CRF gene expression in the paraventricular nucleus

Our results suggest an age-related rise in the endogenous gene expression of CRF in rats until 18 months of age. Our observations are in accord with several previous studies demonstrating maintained or even increased CRF expression in old age [Bao and Swaab 2007], [Aguilera 2011]. These findings support the potential contribution of endogenous CRF effects to aging anorexia but not to middle-aged obesity.

6. SUMMARY OF NOVEL FINDINGS

I. Novel findings of acute experiments:

- Anorexigenic effects of ICV CRF injections declined with aging in both male and female Wistar rats, although CRF-induced anorexia remained significant in all female groups.
- Regarding hypermetabolic effects, female and male rats appear to share the tendency for age-related decline in the responsiveness to acute central CRF administration, nevertheless all age-groups of male animals showed significant hypermetabolic responsiveness.
- Only age-related decline in the hypermetabolic responsiveness to central CRF injection proved to be similar to the effects of central leptin injections. Other CRF-induced changes differed from the effects of central alpha- MSH or leptin injections.
- The maintained anorexigenic efficacy of CRF in females may contribute to their lack of middle-aged obesity and may possibly promote later weight loss in older female animals. In males, no such association may be observed.
- However, hypermetabolic effects declined with aging in both males and females. The high remaining level of CRF-induced hypermetabolism of old male rats—may contribute to their age-related weight loss.

II. Novel findings of chronic experiments

- Chronic ICV CRF administration induced pronounced anorexia and BW loss in young adult, aging and old male rats in contrast with middle-aged animals.
- Central CRF infusion did not show any remarkable age-related changes in hypermetabolic/hyperthermic effects.
- Unlike hyperthermia/hypermetabolism, CRF-induced anorexia and consequent weight loss, appears to show similar age-related patterns as those previously described in case of chronic central melanocortin and leptin administrations.
- With regard to our hypothesis, our results confirm the potential contribution of age-related changes in the anorexigenic responsiveness to a CRF infusion to aging anorexia and consequent weight loss in the old age-groups.

III. Novel findings of the investigation of CRF gene expression in the PVN:

- In the PVN, CRF gene expression increased with aging until 18 months with a subsequent slight decline in the 24-month group.
- Age-related changes in CRF gene expression in the PVN may contribute to the phenomena of aging anorexia.

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9. LIST OF PUBLICATIONS AND PRESENTATIONS

9.1. Articles related to the thesis

1. **Judit Tenk**, Ildikó Rostás, Nóra Füredi, Alexandra Mikó, Szilvia Soós, Margit Solymár, Balázs Gaszner, Diána Feller, Miklós Székely, Erika Pétervári, Márta Balaskó: Acute central effects of corticotropin- releasing factor (CRF) on energy balance: effects of age and gender. *Peptides*. 2016;85:63-72. **IF: 2.778**
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6. Alexandra Mikó, Nóra Füredi, **Judit Tenk**, Ildikó Rostás, Szilvia Soós, Margit Solymár, Miklós Székely, Márta Balaskó, Susanna Brunner, Barbara Kofler, Erika Pétervári. Acute central effects of alarin on the regulation on energy homeostasis. *Neuropeptides*. 2016;S0143-4179(16):30087-30097. **IF:2.486**

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9.3.Presentations related to the thesis

1. **Tenk J.** A CRF hatása energetikai folyamatokban. IV. Grastyán Endre Szakkollégium Intézményi Konferencia 2010, Pécs
2. **Tenk J.** A corticotropin- releasing factor életkorfüggő hatásai az energia háztartás egyes paramétereire PTE ÁOK Házi Tudományos Diákköri Konferencia 2011, Pécs
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8. **Tenk J**, Mikó A, Füredi N, Lőrincz O: Age- associated alterations in corticotropin effects on energy homeostasis are disparate. CROSS, 8th International Biomedical Croatian Student Summit 2012, Zágráb
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9.4. Other presentations

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5. Füredi N, **Tenk J**, Mikó A. Age-associated alterations in cholecystokinin effects concerning energy balance are disparate. CROSS, 8th International Biomedical Croatian Student Summit 2012, Zágráb

6. Mikó A, Füredi N, **Tenk J**. Complex changes in the energy homeostasis of spontaneously hypertensive rats (SHR). CROSS, 8th International Biomedical Croatian Student Summit 2012, Zágráb
7. Füredi N, **Tenk J**. Disparate age-related alterations in cholecystokinin (CCK) effects on energy homeostasis. 5th HMAA, Balatonfüred Amerikai Magyar Orvosszövetség Konferenciája 2012, Balatonfüred
8. Füredi N, Mikó A, **Tenk J**. Complex changes in energy homeostasis of spontaneously hypertensive rats (SHR). 22nd Annual BioCity Symposium 2012, Turku
9. Füredi N, **Tenk J**, Rostás I. Central and peripheral effects of cholecystokinin (CCK) on food intake during the course of aging. János Szentágothai Memorial Conference and Student Competition 2012, Pécs
10. Rostás I, Füredi N, **Tenk J**, Mikó A. Thermoregulatory effects of alpha-melanocyt-stimulating hormone depend on age. János Szentágothai Interdisciplinary Conference and Student Competition 2012, Pécs
11. Mikó A, Füredi N, Rostás I, **Tenk J**. Metabolic adaptation to food deprivation in spontaneously hypertensive rats (SHR). János Szentágothai Memorial Conference and Student Competition 2012, Pécs
12. Mikó A, **Tenk J**, Füredi N. Orexigén és anorexigén neuropeptidok hatásának vizsgálata spontán hipertenzív patkányokban. PTE ÁOK TDK Konferencia 2012, Pécs
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15. Mikó A, Rostás I, **Tenk J**, Füredi N. Spontaneously hypertensive rats (SHR) show complex changes in their energy homeostasis. LIMSC- Leiden International Medical Student Conference 2013, Leiden
16. Rostás I, Mikó A, Füredi N, **Tenk J**. Complex effects of neuropeptide alpha-melanocyt-stimulating hormone on energy homeostasis during the course of aging. LIMSC- Leiden International Medical Student Conference 2013, Leiden
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