Age-related changes in central effects of corticotropin-releasing factor (CRF) suggest a role for this mediator in aging anorexia and cachexia

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Abstract Hypothalamic corticotropin-releasing factor (CRF) lays downstream to catabolic melanocortins and at least partly mediates their catabolic effects. Age-related changes in the melanocortin system (weak responsiveness in middle-aged and a strong one in old rats) have been shown to contribute to middle-aged obesity and later to aging anorexia and cachexia of old age groups. We hypothesized that catabolic (anorexigenic and hypermetabolic) CRF effects vary with aging similarly to those of melanocortins. Thus, we aimed to test whether age-related variations of CRF effects may also contribute to middle-aged obesity and aging anorexia leading to weight loss of old age groups. Food intake, body weight, core temperature, heart rate, and activity were recorded in male Wistar rats of young, middle-aged, aging, and old age groups (from 3 to 24 months) during a 7-day intracerebroventricular CRF infusion (0.2 µg/µl/h) in a

biotelemetric system. In addition, CRF gene expression was also assessed by quantitative RT-PCR in the paraventricular nucleus (PVN) of intact animals of the same age groups. The infusion suppressed body weight in the young, aging, and old rats, but not in middle-aged animals. Weak anorexigenic and hypermetabolic effects were detected in the young, whereas strong anorexia (without hypermetabolism) developed in the oldest age groups in which post mortem analysis showed also a reduction of retroperitoneal fat mass. CRF gene expression in the PVN increased with aging. Our results support the potential contribution of age-related changes in CRF effects to aging anorexia and cachexia. The role of the peptide in middle-aged obesity cannot be confirmed.

Keywords Corticotropin-releasing factor (CRF) · Energy homeostasis · Food intake · Core temperature · Aging · Wistar rat

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Introduction

Corticotropin-releasing factor (CRF) is a 41-aa neuropeptide (Vale et al. 1981) that is produced predominantly in the parvocellular neurons of the paraventricular nucleus of the hypothalamus (PVN). Synthesis and expression of messenger RNA (mRNA) of the peptide have also been detected in the cerebral cortex, in the

amygdala, and also in the hippocampus among other brain areas (Morin et al. 1999; Wang et al. 2011).

This peptide participates in a great variety of processes in humans and also in laboratory rodents including mediation of stress response mainly via activation of the hypothalamic-pituitary-adrenal (HPA) axis (Rivier and Vale 1983; Muglia et al. 1995) and that of the sympathetic tone (Brown et al. 1982). This mediator has also been shown to induce fear and to be involved in mood and anxiety disorders in humans via affecting limbic brain regions (Wasserman et al. 2010; Kormos and Gaszner 2013). In addition, experiments in mice and rats show that upon central administration, CRF elicits coordinated catabolic effects, i.e., it induces anorexia (Rivest et al. 1989; Rothwell 1990) and hypermetabolism accompanied by increased brown fat thermogenesis (Brown et al. 1982; LeFeuvre et al. 1987; Carlin et al. 2006). Moreover, CRF was demonstrated to prevent weight gain in genetically obese rats (Rohner-Jeanrenaud et al. 1989). Regarding catabolic effects, CRF acts downstream to catabolic melanocortins (Lu et al. 2003; Valassi et al. 2008) and, at least partly, mediates their catabolic effects (Tachibana et al. 2007; Kawashima et al. 2008). Previous studies demonstrated that age-related changes in the melanocortin system [weak responsiveness in middle-aged and a strong one in old rats to melanocortin agonist alpha-melanocyte-stimulating hormone (alpha-MSH)] may contribute to middle-aged obesity and later to aging anorexia and cachexia of old age groups (Pétevári et al. 2010, 2011a; Rostás et al. 2015).

Predominantly to CRF1 (CRF1R) and to a lesser extent to CRF2 receptors (CRF2R) (Vaughan et al. 1995; Liaw et al. 1997; Perrin and Vale 1999; Reul and Holsboer 2002) mediate the effects of CRF. These receptors belong to class B of the family of seven-transmembrane G-protein-coupled receptors, sharing 65–68% overall homology at the amino acid level (Vaughan et al. 1995; Chatzaki et al. 2004).

Widespread expression of CRF1R has been reported in the central nervous system including the anterior pituitary, the hypothalamic nuclei, and the cerebral cortex (Potter et al. 1994; Van Pett et al. 2000; Reul and Holsboer 2002; Justice et al. 2008). This receptor type was shown to promote anxiogenic and depressive behavior (Van Pett et al. 2000; Reul and Holsboer 2002) and hyperthermia (Figueiredo et al. 2010). Its moderate

anorexigenic effects have been attributed to emotional stress (Hotta et al. 1999).

On the other hand, expression of CRF2R is more restricted. Among other locations, such receptors were detected in the amygdala, in the hippocampus, in the hypothalamic nuclei (e.g., ventromedial hypothalamus) (Van Pett et al. 2000), and in the nucleus of the solitary tract (Bittencourt and Sawchenko 2000). Various studies established a primary role of CRF2R in mediating the anorexigenic actions of central CRF administration (Cullen et al. 2001; Stengel and Taché 2014). In addition, CRF2Rs are thought to mediate anxiolytic, antidepressive behavior (Van Pett et al. 2000; Reul and Holsboer 2002).

During the course of aging, long-term trends emerge in the regulation of energy balance resulting in middle-aged obesity and aging anorexia leading to loss of active tissues and cachexia and sarcopenia in old age (Scarpace et al. 2000; Morley 2001; Di Francesco et al. 2007; Pétevári et al. 2011b; Sertié et al. 2015; Tay et al. 2015; Wysokiński et al. 2015; Jura and Kozak 2016; Loenneke and Loprinzi 2016). Both abnormalities represent serious public health challenges (Morley 2001; Sertié et al. 2015; Sivasinprasasn et al. 2015; Wysokiński et al. 2015; Jura and Kozak 2016). The question arises whether production or efficacy of catabolic CRF show changes in the course of aging that may potentially contribute to the above-mentioned age-associated obesity and/or to the development of sarcopenia.

Most studies on animals and in humans have reported increased hypothalamic CRF expression (with compensatory CRF1R downregulation) during aging (Scaccianoce et al. 1990; Tizabi et al. 1992; Ceccatelli et al. 1996; Bao and Swaab 2007; Aguilera 2011). Nevertheless, some observations in aging Fisher rats described unchanged or even reduced CRF expression (Cizza et al. 1994; Kasckow et al. 1999).

We hypothesized that catabolic (i.e., anorexigenic and hypermetabolic) CRF effects vary with aging similarly to those of melanocortins. Thus, we aimed to test 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.

To test this hypothesis, in the present study, we aimed to analyze age-related changes in the CRF gene expression in the PVN and anorexigenic and hypermetabolic responsiveness to a 7-day intracerebroventricular (ICV) CRF infusion in different age groups of male Wistar rats.

Materials and methods

Animals

In the present study, young adult, middle-aged, aging, and old male Wistar rats (3, 12, 18, and 24 months of age, respectively) were used from the colony of the Institute for Translational Medicine, Medical School, University of Pécs, Hungary. Mean body weights (BW) of control vs. CRF-treated animals of these age groups were as follows: 3 months 366.9 ± 13.5 g vs. 348.4 ± 10.62 g, 12 months 526.3 ± 29.0 g vs. 516.4 ± 31.1 g, 18 months 479.5 ± 22.5 g vs. 460.0 ± 19.4 g, and 24 months 445.9 ± 14.4 g vs. 450.4 ± 14.2 g. When they reached the appropriate age, rats were housed individually in plastic cages (375×215 mm, height 149 mm, equipped with feeder and bottle container) with wood chip bedding and a steel grid cover. The animals were kept at an ambient temperature of 24–25 °C (thermoneutrality in the nest) under conditions of controlled illumination (with 12:12-h dark-light regime, lights were on from 06:00 a.m.). Animals had free access to powdered standard laboratory rat chow (CRLT/N rodent chow, Szindbád Kft., Gödöllő, Hungary, 11 kJ/g) and tap water. Spontaneous daily food intake (FI) and BW were measured every day; consequently, the animals were habituated to regular handling. All experimental interventions and procedures were undertaken according to the general rules and following the special permission of the University of Pécs Ethical Committee for the Protection of Animals in Research (BA 02/2000-11/2011, valid for 5 years). In general, the rules of this committee are in good accord with the main directives of the European Communities Council (86/609/EEC, Directive 2010/63/EU of the European Parliament and of the Council).

Surgeries

After at least 1 week of adaptation to the biotelemetric system (MiniMitter-VMFH series 4000, Sunriver, OR) and 5–7 days before the start of the infusion, an e-mitter was implanted intraperitoneally (IP) under ketamine + xylazine [78 mg/kg (Calypsol, Richter) + 13 mg/kg (Sedaxylan, Eurovet)] anesthesia, which provided 1–1.5-h deep narcotic state. Following surgical interventions, IP gentamycin injection was also applied to avoid infections.

After full recovery from the e-mitter implantation, an ICV cannula (Alzet, BrainKit) was stereotaxically implanted into the right lateral cerebral ventricle (coordinates according to Paxinos and Watson 2006) under similar anesthesia. Simultaneously, an Alzet osmotic minipump was inserted subcutaneously underneath the nape of the neck which was attached to the BrainKit. The minipump was filled with CRF dissolved in pyrogen-free saline (PFS) or PFS and provided the delivery of these substances at controlled rates (1 μ l/h) for 7 days.

Biotelemetric and other measurements

E-mitter implantation was performed according to our previous studies (Soós et al. 2010; Pétervári et al. 2011a). The intraperitoneally implanted e-mitter detected core temperature (T_c), heart rate (HR, representing metabolic rate; Butler 1993), and spontaneous horizontal locomotor activity (Act) and sent signals about these parameters to the receiver, which was placed under the MiniMitter chamber. The biotelemetric system registered data every 5 min that were integrated into 12-h periods (two mean values per day), equivalent to the daily inactive and to the nighttime active phase. For primary data analysis, the VitalView software provided by the manufacturer (MiniMitter) was used. Data recorded on the day of the implantation of the osmotic minipump and ICV cannula and also on the following night were excluded from the analysis because of the inflammatory response induced by the surgical procedures (Buwalda et al. 1997). In this system, FI and BW were measured manually daily.

Substances applied

Corticotropin-releasing factor (CRF-41, Bachem, AG Switzerland) dissolved in PFS and PFS alone (as control) were delivered into the right lateral cerebral ventricle via Alzet osmotic minipumps at a flow rate of 1 μ l/h for 7 days. The applied dose of CRF (0.2 μ g/ μ l/h) was chosen according to earlier observations (Rivest et al. 1989).

Sampling, RNA isolation, and quantitative real-time polymerase chain reaction

Intact normally fed male Wistar rats of each age group ($n = 6$ –7/group) were decapitated. The brains were quickly dissected, frozen in liquid nitrogen, and stored

at -70°C . PVN samples were punched from 1-mm thick slices (-2 to -3 mm from the Bregma; Paxinos and Watson 2006) of the brains cut on a brain matrix (Ted Pella, CA, USA) by two razor blades. Sections were placed on a chilled mat, and the mediobasal hypothalamic area containing the PVN was microdissected by a 1-mm-diameter Harris punching needle (Sigma-Aldrich, Budapest, Hungary).

The total RNA was isolated with the Pure Link™ RNA Mini Kit (Life Sciences, Carlsbad, CA, USA) according to the protocol suggested by the manufacturer. Samples were homogenized; RNA was purified by ethanol treatment and eluted from the membrane. The total amount of RNA was determined by NanoDrop (Thermo Scientific). High-capacity complementary DNA (cDNA) kit was applied (Applied Biosystems, Foster City, CA, USA) to perform cDNA synthesis, using $1\text{ }\mu\text{g}$ of total RNA sample according to the official protocol.

For CRF gene expression analysis, quantitative real-time polymerase chain reaction (qRT-PCR) was performed using SensiFast SYBR Green reagent (BioLine). Amplifications were run on ABI StepOnePlus system. StepOne software was used to analyze gene expression, which was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene. Based on the quality of previous PCR reference curves in male Wistar rats and other rodents, the GAPDH was chosen as a reference gene (Pibiri et al. 2015; Füredi et al. 2016). The primer sequences are shown in Table 1. PCR conditions were also set according to previous studies (Füredi et al. 2016): 1 cycle 95°C for 2 min and 40 cycles at 95°C for 5 s and 60°C for 30 s. The amplification of PCR products were calculated according to the $2^{-\Delta\Delta\text{Ct}}$ method.

Postmortem examination

After the ICV infusion, animals were euthanized by an overdose of urethane ($3\text{--}5\text{ g/kg}$ BW, IP, Reanal), and the

injection sites were checked macroscopically by coronal sections of the removed and fixed brains in all cases. Data of rats with inappropriately placed cannula were excluded from the analysis. The retroperitoneal and epididymal fat pads of the animals were removed and weighed, along with the tibialis anterior muscle, as indicators of body composition.

Statistical analysis

Each animal group contained at least six to eight rats. All results are shown as mean $\pm$ SEM. For statistical analysis of the data, repeated measures ANOVA and one-way ANOVA with Tukey's post hoc test were applied using SPSS for Windows 11.0 software. The significance was set at the level of $p < 0.05$.

Results

Body composition values (calculated for 100 g BW) and mean BWs of different age groups (Table 2) 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 ($p < 0.05$). Concerning body composition indicators, epididymal fat values were found to be different between young adult (3-month) rats vs. 18 or 12-month groups and between the 12 or 18 vs. 24-month group, whereas retroperitoneal fat pad of young adult rats differed significantly from values of 18-month, aging animals ($p < 0.05$). No difference in muscle mass was detected in any group, except for the oldest (24-month) sarcopenic animals ($p < 0.05$ for rats 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 (as shown by repeated measures ANOVA comparing the curves of treated vs. control rats) in the 3, 18, and 24-month age groups, but not in middle-aged (12-month) animals [3 months $F(1, 14) = 10.548$, $p = 0.006$; 18 months $F(1, 10) = 23.436$, $p = 0.001$; 24 months $F(1, 14) = 44.239$, $p < 0.001$] (Fig. 1). By the end of the CRF infusion, significant reduction of retroperitoneal fat developed in the oldest groups [18 months $p = 0.009$; 24 months 0.036 , one-way ANOVA], while no change occurred in epididymal fat or muscle mass in any group (Table 2).

Table 1 Primer sequences of the tested corticotropin-releasing factor (CRF) gene and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) housekeeping gene

Primers	Forward	Reverse
CRF	CCGGGCAGAGCAGT TAGC	CAACATTTTCATTTT CCGATAATCT
GAPDH	TGCCATCACTGCCA CTCAGA	GTCAGATCCACAAC GGATACATTG

Table 2 Indicators of body composition at the end of a 7-day intracerebroventricular corticotropin-releasing factor (CRF) or pyrogen-free saline (PFS) infusion in different age groups of rats

Group (age and nutritional state)	Epididymal fat (g/100 g body weight)		Retroperitoneal fat (g/100 g body weight)		Tibialis anterior muscle (g/100 g body weight)	
	PFS	CRF	PFS	CRF	PFS	CRF
3 months	0.35 ± 0.04 ^a	0.43 ± 0.05	0.29 ± 0.04 ^c	0.41 ± 0.06	0.16 ± 0.01	0.18 ± 0.01
12 months	0.53 ± 0.05	0.46 ± 0.06	0.47 ± 0.07	0.49 ± 0.08	0.19 ± 0.01	0.17 ± 0.04
18 months	0.55 ± 0.01	0.47 ± 0.05	0.60 ± 0.13	0.32 ± 0.06 ^c	0.18 ± 0.01	0.18 ± 0.01
24 months	0.39 ± 0.03 ^b	0.46 ± 0.02	0.44 ± 0.05	0.28 ± 0.03 ^c	0.15 ± 0.02 ^d	0.17 ± 0.01

^a Among control, PFS-treated animals, epididymal fat pads of 12 and 18-month age groups differ significantly from values of 3-month-old animals

^b Among control, PFS-treated animals, epididymal fat pads of 12 and 18-month age groups differ significantly from values of 24-month-old animals

^c Significant difference between retroperitoneal fat of 3 vs. 18-month control animals

^d Significant difference between muscle indicator values of 12 vs. 24-month animals

^e Significant difference between corresponding retroperitoneal fat values of CRF-treated vs. PFS-treated 18 and 24-month rat groups

Concerning the anorexigenic effects, the CRF infusion elicited the strongest suppression during the first 2 days in all rats. Significant anorexia was detected for 2 days in the 3-month and for 7 days in the 18 and 24-month animals [3 months $F(1, 14) = 7.430$, $p = 0.016$; 18 months $F(1, 10) = 6.803$, $p = 0.028$; 24 months $F(1, 14) = 19.771$, $p = 0.001$, repeated measures ANOVA] (Fig. 2). Figure 3 describes 7-day cumulative energy intake in CRF-treated vs. respective control groups demonstrating the age dependence and short-term feature of CRF-induced anorexia (18 months $p = 0.035$; 24 months $p < 0.001$, one-way ANOVA). Accordingly, anorexigenic effects of the CRF infusion were strongest in the oldest rats.

Our present data demonstrate that mean nighttime control HR values of young adult rats differed from those of older age groups [3 vs. 12, 18, 24 months $F(1, 23) = 7.808$, $p = 0.001$, repeated measures ANOVA, p values of post hoc analysis 3 vs. 12 months 0.002, 3 vs. 18 months 0.025, 3 vs. 24 months 0.002] (Fig. 4). 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 (Pétevári et al. 2014). During the ICV CRF infusion, mean daytime HR (inactive period, nadir of the circadian rhythm) failed to show significant increase during the infusion. The slight rise in HR in middle-aged and old rats on day 1 of the infusion is likely to be attributable to the surgery.

Mean basal (preinfusion) daytime 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: [3 months $F(1, 12) = 7.836$, $p = 0.016$, repeated measures ANOVA] (Fig. 5) in young adult rats. The slight rise in the mean daytime T_c value of day 1 in aging animals is probably due to the surgery, while differences in other groups did not reach statistical significance.

Spontaneous nighttime horizontal locomotor activity 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 activity was detectable (Fig. 6).

In our study, qRT-PCR measurements revealed that in the PVN of different age groups of rats, CRF mRNA expression increased with aging until 18 months ($p = 0.036$, one-way ANOVA) with a subsequent slight decline in the 24-month animals. Post hoc analysis showed significant difference between the young and aging groups (3 vs. 18-month rats $p = 0.006$). In addition, a rising tendency was observed between the middle-aged and aging groups (12 vs. 18-month animals $p = 0.051$) (Fig. 7).

Discussion

The objective of the present study was to test the hypothesis that age-related changes in CRF gene expression in the PVN and in the catabolic (i.e., anorexigenic

Fig. 1 Changes of body weight (ΔBW) prior to and during the course of a 7-day intracerebroventricular infusion of pyrogen-free saline (control, open symbols) vs. corticotropin-releasing factor (CRF, closed symbols) in rats of different age groups. Asterisks indicate significant differences ($*p < 0.05$) determined by repeated measures ANOVA

and hypermetabolic) CRF effects may contribute to middle-aged obesity and aging anorexia and consequent weight loss of old age groups.

In the PVN, CRF gene expression increased with aging until 18 months with a subsequent slight decline in the 24-month group (Fig. 7). These results suggest an age-related rise in the endogenous gene expression of CRF in rats. These findings support the potential

Fig. 2 Daily food intake (FI) values prior to and during the course of intracerebroventricular infusion of pyrogen-free saline (control, open bars) vs. corticotropin-releasing factor (CRF, closed bars) in rats of different age groups. The fall at day 0 was due to the surgical intervention. Asterisks indicate significant differences ($*p < 0.05$) in the variables during the indicated period between the CRF-treated vs. control groups, as determined by repeated measures ANOVA

contribution of endogenous CRF effects to aging anorexia but not to middle-aged obesity.

Fig. 3 Cumulative food intake (FI in kJ) values from day 1 to day 7 in the course of an intracerebroventricular infusion of pyrogen-free saline (control, open bars) or corticotropin-releasing factor (CRF, closed bars). Asterisks indicate significant differences ($*p < 0.05$) between CRF-treated vs. pyrogen-free saline-treated (control) rats as determined by one-way ANOVA. Above the bars, the rates of CRF-induced suppression (the difference between values of corresponding control and CRF-treated groups expressed as percentage of the control energy intake) are also indicated

Fig. 4 Heart rate (HR) values averaged for 12-h periods before and during the course of an intracerebroventricular infusion of pyrogen-free saline (control, *gray lines*) or corticotropin-releasing factor (CRF, *black lines*) in rats of different age groups. The mean daytime (inactive phase) values are represented by *open symbols*, the mean nighttime HR values by *closed symbols*. The *white-black bars* underneath the abscissa refer to the day-night periods, respectively

Concerning responsiveness to exogenous CRF, our results regarding the effects of the CRF infusion on parameters of energy balance in young adult rats were in accord with previous observations (Rivest et al. 1989; Buwalda et al. 1997). Compared with the weak, but significant CRF-induced BW reduction in young adult rats, the oldest animals (18, 24 months) showed strong weight loss during the course of the infusion (Fig. 1).

Middle-aged animals failed to lose weight. Accordingly, at the end of the infusion, post mortem body composition indicators demonstrated the biggest changes in the two oldest age groups: Retroperitoneal fat mass was found to be reduced in CRF-treated animals, whereas epididymal fat did not show any decline (Table 2). Although CRF infusion-induced decreases in fat mass were reported previously by other researchers in young age groups of

Fig. 5 Core temperature (Tc) values averaged for 12-h periods before and during the course of an intracerebroventricular infusion of pyrogen-free saline (control, *gray lines*) or corticotropin-releasing factor (CRF, *black lines*) in rats of different age groups. The mean daytime (inactive phase) values are represented by *open symbols*, the mean nighttime Tc values by *closed symbols*. The

white-black bars underneath the abscissa refer to the day-night periods, respectively. The deep fall in Tc at day 0 in all age groups was due to the surgical intervention. Asterisk indicates the significant difference for mean daytime body temperature values of 3-month control vs. age-matched treated rats for days 2 and 3 (* $p < 0.05$) determined by repeated measures ANOVA

Fig. 6 Activity (Act) values averaged for 12-h periods before and during the course of an intracerebroventricular infusion of pyrogen-free saline (control, gray lines) or corticotropin-releasing factor (CRF, black lines) in rats of different age groups. The mean daytime (inactive phase) values are represented by open symbols, the mean nighttime Act values by closed symbols. The white-black bars underneath the abscissa refer to the day-night periods, respectively. The falls at day 0 (both for the daytime and nighttime values) were due to the surgical intervention

different rat strains (Arase et al. 1988; Cullen et al. 2001), in our study, no change in fat mass indicators was detected either in young adult or in middle-aged groups. Muscle mass indicators did not show any CRF-induced or PFS-induced change in any group (Table 2), indicating a lack of sarcopenic effects of our 7-day CRF infusion. This lack of sarcopenia (a typical consequence of overactivity of glucocorticoids; Millward et al. 1976; 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; Zambrano et al. 2015).

With regard to CRF-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 (Figs. 2 and 3). The above-described findings strongly support the contribution of age-associated alterations in anorexigenic responsiveness to CRF in aging anorexia and cachexia, and they do not contradict a potential role of these changes in middle-aged obesity. A number of factors may play a role in the enhanced anorexigenic effects of CRF in old age groups. We propose that (1) similar age-related patterns characterizing activators of CRF such as melanocortin agonist α -MSH or adipose tissue-derived leptin (an activator of the melanocortin system, see Fig. 8), (2) age-related diminishment of glucocorticoid release (antagonizing central anorexigenic CRF effects) from the adrenal cortex of old rats upon adrenocorticotropin (ACTH) activation (Rebuffat et al. 1992), and (3) age-related decline in the activity of antagonistic orexigenic/anabolic mediator systems such as that of neuropeptide Y (Sahu et al. 1988) may be also found in the background.

Hypermetabolic effects of the CRF infusion were weak and did not show remarkable age-related variations

Relative expression of CRF mRNA in the PVN

Fig. 7 Relative mRNA expression of corticotropin-releasing factor (CRF) gene by quantitative RT-PCR in the paraventricular nucleus (PVN) in different age groups of male Wistar rats. Data were evaluated by the delta delta Ct method for qRT-PCR analysis. CRF mRNA expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression. Asterisk indicates significant difference between 3 and 18-month animals

Fig. 8 A proposed scheme depicting the complexity of the regulation of energy balance with special emphasis on the activation route of corticotropin-releasing factor (CRF). Effects known to be mediated via CRF type 1 (CRF1R) or via CRF type 2 receptors (CRF2R) that are also potentially relevant for the interpretation of

our data are also shown. *alpha-MSH* alpha-melanocyte-stimulating hormone, *AgRP* agouti-related peptide, *CART* cocaine-amphetamine-regulated transcript, *NPY* neuropeptide Y, *POMC* pro-opiomelanocortin, *PVN* paraventricular nucleus of the hypothalamus

(Fig. 4). A transient moderate CRF-induced hyperthermia was observed in the young adult group, but no change of T_c (daytime or nighttime) developed in middle-aged or older rats (Fig. 5). Apart from some surgical procedure-induced suppression, no significant alteration of spontaneous activity was detected in any age group (Fig. 6).

In summary, the age-related pattern of alterations in CRF anorexia and consequent weight loss appears to be similar to that previously described in case of central catabolic melanocortin effects (Pétevári et al. 2011a), i.e., significant responsiveness in the young adult, followed by a suppression of catabolic effects in the middle-aged and a recovery of efficacy in the old age groups. Unlike hyperthermia/hypermetabolism induced by ICV infusions of α -MSH (Williams et al. 2011), where increases of corresponding parameters (T_c and HR) were strong in young adult and middle-aged rats and declined thereafter, hyperthermic effects of CRF appeared only in the young adult animals and failed to

develop in the older groups (Pétevári et al. 2011a). In addition, increase of HR failed to reach a significant extent in any age group. Thus, hypermetabolic effects of the 7-day 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 (Pétevári et al. 2011a).

With regard to our hypothesis, our results confirm the potential contribution of age-related changes in CRF gene expression in the PVN and those in the anorexiogenic responsiveness to a CRF infusion to aging anorexia and consequent weight loss in the old age groups. No such evidence was found for middle-aged obesity.

Conclusions and future perspectives

Despite our positive results, our study had numerous limitations. Acute effects of the CRF infusion could

not be assessed in our experimental model, since the infusion started already 10–12 h following the implantation of the osmotic minipump. Therefore, effects of surgery influenced data recorded during the first day of the infusion. In addition, there is a lack of internal positive controls in this study. In case of middle-aged (12-month) rats, CRF infusion failed to elicit anorexia, weight loss, or hyperthermia. However, our previous reports demonstrate that in similar middle-aged male Wistar rats, a similar 7-day ICV infusion of a melanocortin agonist alpha-MSH successfully induced hyperthermia for 4–5 days. The duration of the alpha-MSH-induced increase in HR also reached 5 days (Pétevári et al. 2011a). A 7-day ICV leptin infusion was also capable of increasing Tc and HR of 12-month male Wistar rats (Pétevári et al. 2014). Concerning anorexigenic responsiveness, a 7-day ICV leptin infusion successfully elicited significant anorexia in this age group resulting in a 40% reduction in overall energy intake (Pétevári et al. 2014). Another important question that could not be addressed by the present study (due to technical obstacles) involves age-related changes in CRF1R and CRF2R systems in the hypothalamus. The role of CRF2Rs promises to be more important from the point of view of this topic, as anorexigenic, but not hypermetabolic responsiveness to CRF seems to be involved in age-related body weight changes. Unfortunately, as yet, no specific CRF2R antibody is available (probably due to the high rate of homology of CRF receptor subtypes; Chatzaki et al. 2004; Lukkes et al. 2011). In the future, the role of CRF2Rs in aging anorexia should also be tested with the help of specific agonists (e.g., urocortin 2 or urocortin 3) or specific antagonists of CRF2R (e.g., antisauvagine-30) (Stengel and Taché 2014). Such investigations may help in the future to identify new targets in the prevention or therapy of aging anorexia.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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