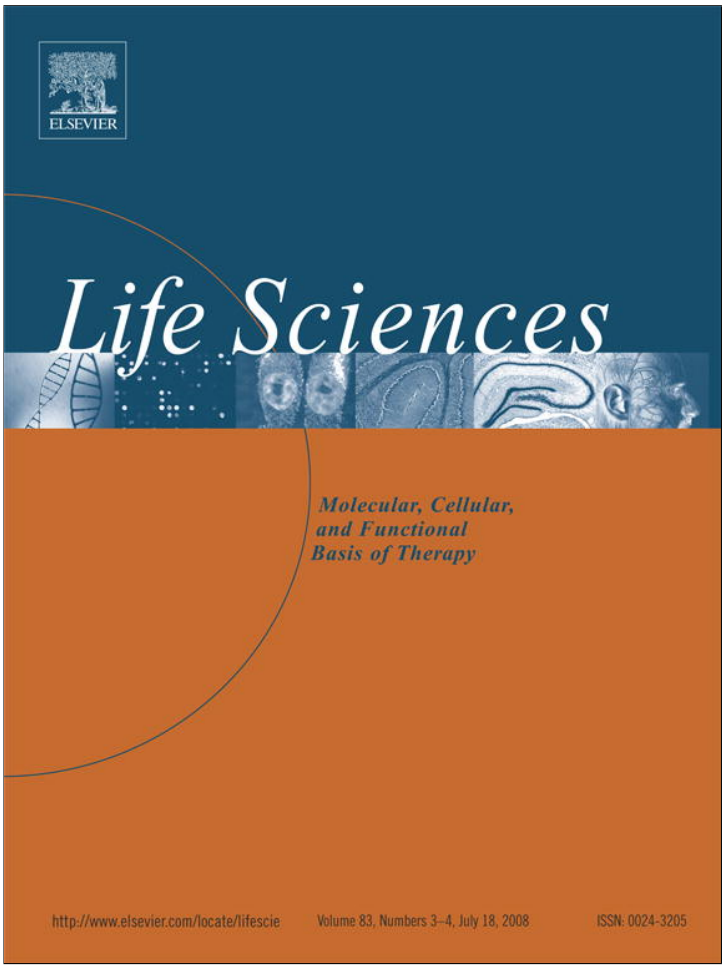

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Effect of aging on 24-hour pattern of stress hormones and leptin in rats

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ABSTRACT

This work analyzes the 24-hour changes of hypothalamic–pituitary–adrenal (HPA) axis activity and leptin release in aged rats. Three- and 22-month-old male Wistar rats were killed at 6 time intervals during a 24-hour cycle (n=8–10 rats/group). Aging augmented plasma ACTH while it decreased plasma and adrenal gland corticosterone levels. Plasma and adrenal corticosterone levels attained high levels during all the scotophase, concomitantly with the maxima in ACTH levels, whereas in aged rats only a brief plasma corticosterone peak at the early scotophase and no time of day variations of adrenal corticosterone were observed. Aging augmented circulating leptin, with a significant interaction "age×time" in the factorial ANOVA, i.e. only in young rats time of day changes were significant, with the lowest values of leptin at the middle of the light period and higher values at night. When plasma leptin was expressed on body weight basis, the age-related differences became not significant but the daily pattern of plasma leptin found in young rats persisted. Plasma and adrenal corticosterone levels correlated significantly with plasma ACTH only in young rats. Likewise, plasma leptin correlated with plasma corticosterone only in young rats. These changes can be attributed to a disrupting effect of aging on the homeostatic mechanisms modulating HPA activity and leptin release.

Introduction

Altered regulation of the hypothalamic–pituitary–adrenal (HPA) axis is typically seen in aged vertebrates ranging from fish to humans (Wexler, 1976) and has been implicated in the acceleration of various age-related diseases (Lupien et al., 1998). There are numerous studies, utilizing various tests, that suggest an impaired glucocorticoid feedback regulation in aged humans (O'Brien et al., 1994; Seeman and Robbins, 1994; Born et al., 1995). However, reports about age-associated changes in the basal activity of the HPA axis in humans are ambiguous: some found the cortisol levels in young and elderly individuals to be similar (Lakatua et al., 1984; Waltman et al., 1991; Born et al., 1995; Gotthardt et al., 1995), others observed age-associated increases in basal plasma cortisol concentrations (Halbreich et al., 1984; Pfohl et al., 1985) while yet others even observed decreased basal cortisol plasma concentrations with aging (Drafta et al., 1982; Sherman et al., 1985; Maes et al., 1994).

Previous studies have often shown activation of the HPA axis or enhanced basal corticosterone levels in aged rats of different strains (Sapolsky et al., 1986a; Sapolsky et al., 1986b; Brodish and Odio, 1989; Dellwo and Beauchene, 1990; Issa et al., 1990; Hauger et al., 1994; Seckl and Olsson, 1995; Sapolsky, 1999; Lucassen and De Kloet, 2001). However, it should be stressed that there are reports failing to find elevated basal corticosterone levels or enhanced stress responses in old rats, or even found decreased glucocorticoid or ACTH levels (Sonntag et al., 1987; Issa et al., 1990; Scaccianoce et al., 1990; van Eekelen et al., 1991; van Eekelen et al., 1992; Morano et al., 1994; Seckl and Olsson, 1995; Cizza et al., 1995; Scaccianoce et al., 1995; Lucassen and De Kloet, 2001; Heine et al., 2004).

Aged rats are resistant to the suppressive effects of dexamethasone (Hatzinger et al., 1996) and an age-related decrease in the sensitivity of corticotropes to glucocorticoids has been documented in vitro (Revskoy and Redei, 2000). This suggested that there is a direct, pituitary-mediated dysregulation of the HPA axis in rats starting as early as in middle age. Behavioral adaptation in aging can become impaired from abnormal expression of corticotropin-releasing hormone and/or its binding protein as shown in 24-month-old Fischer 344 rats (Xiao et al., 2006). These changes may contribute to impaired adaptations to stress and other pathophysiological processes during aging.

It must be noted that several of these studies have been performed only at certain time periods in the 24-hour cycle, a potential drawback in view of the significant 24-hour variations that HPA hormones have. Taking this into account, we undertook the present study to assess whether aging affects the mean levels and 24-hour variations of plasma ACTH, plasma corticosterone and adrenal gland corticosterone concentration. Circulating leptin, an important peripheral hormonal signal in the regulation of energy homeostasis (Yang and Barouch, 2007; Myers et al., 2008; Vickers, 2007; Bluher and Mantzoros, 2007)

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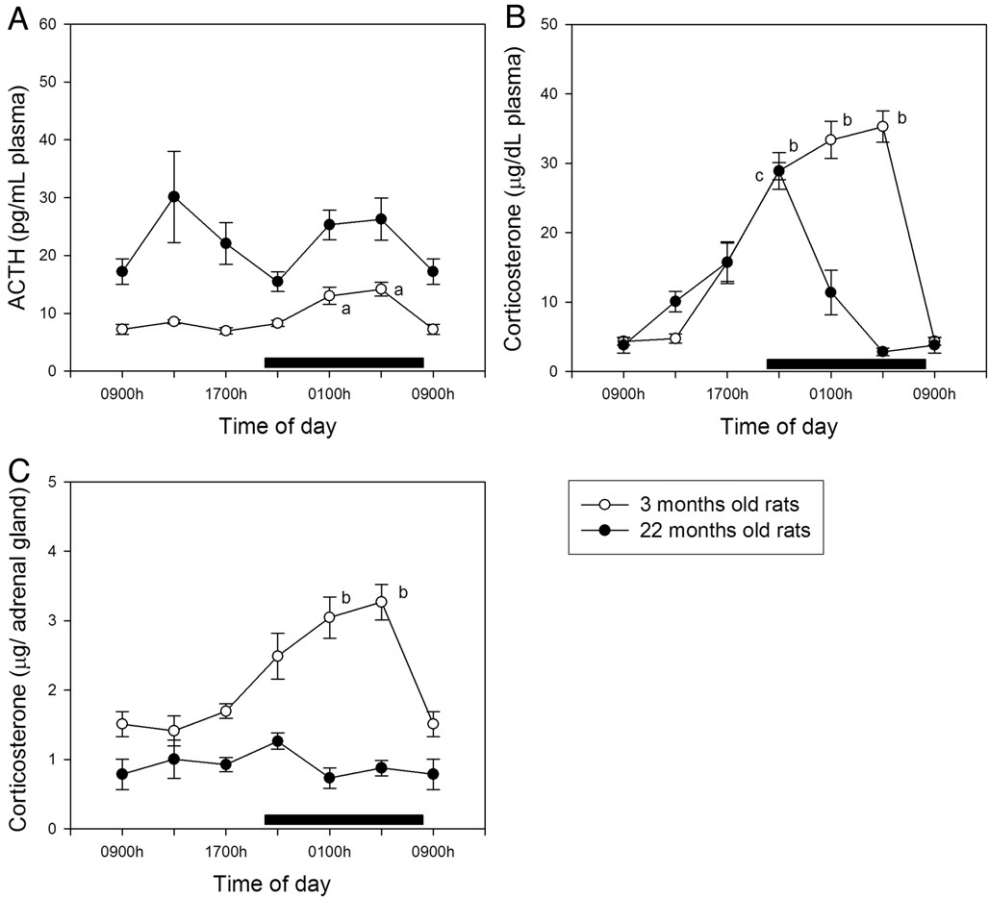

Fig. 1. Twenty-four-hour changes in plasma ACTH (panel A), plasma corticosterone (panel B) and adrenal gland corticosterone (panel C) in young (3 months old) and old (22 months old) rats. Groups of 8–10 rats were killed by decapitation at 6 different time intervals throughout a 24-hour cycle. Bars indicate scotophase duration. Shown are the means±SEM. Letters indicate the existence of significant differences between time points within each age group after a one-way ANOVA followed by a Student–Newman–Keuls multiple comparisons test, as follows: $^{a}p<0.01$ vs. 0900, 1300, 1700 and 2100 h. $^{b}p<0.02$ vs. 0900, 1300 and 1700 h. $^{c}p<0.05$ vs. the remaining time points. For further statistical analysis, see text.

and that displays circadian rhythmicity with maxima during the scotophase and a nadir late at the light phase of daily photoperiod (Xu et al., 1999; Chacon et al., 2005; Perelló et al., 2006) was also measured. Specifically, we sought to answer whether the relationship between the studied hormones persisted in aged rats as an index of homeostasis integrity.

Materials and methods

Animals and experimental design

Three- and 22-month-old male Wistar rats were maintained under standard conditions with controlled light (12:12-hour light/dark schedule; lights on at 08:00 h) and temperature (22±2 °C).

Because we had previously reported that social isolation in rats induced higher plasma glucocorticoid levels than group-caged rats (Perelló et al., 2006), rats were grouped at 4–5 individuals per cage for 1 week before the experiment. Animals were gently handled (holding on each animal for 30 s before transferring it into another cage, daily for week, to minimize stress conditions). On the experimental day, animals were sacrificed by decapitation without previous anesthesia at 6 different time intervals (8–10 rats per time per group), every 4 h throughout a 24-hour cycle, starting at 09:00 h. It should be stated that on the experimental day, the respective experimental-time cages were moved (approximately, 3 and a half hour before sacrifice) to a room next to the general one. The rats were decapitated by one operator, with 30–40-second time interval between animals while another operator immediately collected the blood and a third one was in charge of tissues' dissections. All experiments were conducted in accordance with the guidelines of the International Council for Laboratory Animal Science (ICLAS). Trunk blood was collected and plasma samples were obtained by centrifugation of blood at 1500 ×g for 15 min and were stored at −20 °C until further analysis. Immediately after sacrifice, the adrenal glands were dissected by a dorsal approach and the surrounding adipose tissue was carefully removed. Glands were transferred into tubes containing a small volume (300 μL) of 0.1 M acetic acid and immediately sonicated (2–3 times for 20 s, on an ice bath). Tubes were then centrifuged (10,000 ×g at 4 °C) for 5 min and the supernatants were kept frozen until the measurement of corticosterone concentration (Giovambattista et al., 2000).

Table 1
Summary of factorial ANOVA for data of Fig. 1

Source	*df*	ACTH (pg/mL plasma)		Corticosterone (μg/dL plasma)		Corticosterone (μg/adrenal gland)	
		F	Significance	*F*	Significance	*F*	Significance
Age	1	75.6	<0.001	32.4	<0.001	64.5	<0.001
Time of day	5	2.51	0.042	32.3	<0.001	3.61	0.008
Age×time	5	1.66	0.164	19.9	<0.001	3.41	0.011
Error	101						
		Mean values±SEM					
Young rats		9.8±0.6		22.3±2.7		2.19±0.87	
Old rats		11.3±1.6		17.3±1.9		0.97±0.07	

Hormone measurements

Plasma ACTH levels were measured by a homologous specific double antibody radioimmunoassay (RIA), using materials kindly supplied by the NIDDK's National Hormone and Pituitary Program and by Dr. A. Parlow (Harbor UCLA Medical Center, 1000 West Carson Street, Torrance CA 90509). The intra- and inter-assay coefficients of variation were 6 and 8%, respectively. Sensitivity of the RIA was 40 pg/mL using the NIDDK rat-ACTH-RP-1 (Esquifino et al., 1999).

Plasma and adrenal concentrations of corticosterone were evaluated by a specific RIA reported elsewhere (Spinedi et al., 1991) with a standard curve between 1 and 250 μg/dL and intra- and inter-assay coefficients of variation of 4–6 and 8–10% respectively. Leptin levels were measured by a specific RIA developed in one of our laboratories (Giovambattista et al., 2000), the standard curve ranging from 0.4–50 ng/mL with intra- and inter-assay coefficients of variation of 5–8 and 10–13%, respectively.

Statistical analysis

Statistical analysis of results was performed by an analysis of variance (ANOVA) following a factorial model of two factors, i.e., age and time of day. For the factorial ANOVA, the analysis included assessment of the group effect (i.e. the occurrence of differences in mean values between aged and young rats), of time of day effects (the occurrence of daily changes) and of the interaction between the two factors (age and time, from which inference about differences in timing and amplitude could be obtained). A post-hoc Tukey's multiple comparison test was applied when one or the two factors, or their interaction, was significant. The two-way ANOVA was chosen because it is capable of analyzing, at the same time, the effects of the age group (i.e. whether means differ in young and old aged groups), the time of day (i.e. whether there is a significant difference between distinct time points) and their interaction (i.e. whether any possible difference between young and old rats is homogeneous during the day or not). This procedure kept the experiment-wise alpha error low thus preserving the comparison power.

One-way ANOVAs followed by Student–Newman–Keuls multiple comparisons tests were employed to show which time points were significantly different within each experimental group to define existence of peaks. Validity of linear models was assessed by residual analysis and Shapiro Wilk's and Bartlett's tests. The results found fit normality and homogeneity of variance. Curve estimation in regression analysis was made by using SPSS software, version 10.1 (SPSS Inc., Chicago, ILL, USA). Samples from animals collected at different time points were included on the assumption that assessment of hormone levels throughout the day would give a reliable definition of correlation (Perelló et al., 2006). p values lower than 0.05 were considered evidence for statistical significance.

Results

The weights of the 3- and 22-month-old rats at the time of sacrifice were 290.9±3.7 and 595.3±10.6 g, respectively (mean±SEM). The effect of aging on the daily secretory pattern of stress-related hormones is shown in Fig. 1. Panel A shows the 24-hour changes in plasma ACTH release. In a factorial ANOVA (Table 1) both age and time of day were identified as significant factors, aging augmenting plasma ACTH significantly. In young rats, the pattern found was characterized by low levels during the day and an increase to peak values at the middle of the scotophase. In aged rats, time of day variation of plasma ACTH was not statistically significant (Fig. 1, panel A).

Fig. 1 (panels B and C) shows the effect of aging on the 24-hour pattern of plasma and adrenal gland corticosterone levels. In aged rats, a significant decrease of plasma and adrenal corticosterone levels was found, as shown by main factor analysis in the factorial ANOVA (Table 1). Time of day changes were significant in the factorial ANOVA for both parameters (Table 1) with significant interactions "age×time of day", i.e., plasma and adrenal corticosterone attained high levels across all time points of the scotophase and paralleled the maxima in ACTH levels. By contrast, aged rats showed only a brief plasma corticosterone peak in the early scotophase (Fig. 1, panels A and B). Adrenal corticosterone did not exhibit any significant time of day variation (Fig. 1, panel C and Table 1).

Fig. 2 depicts the changes in circulating leptin levels in young and aged rats. When expressed as ng/mL of plasma (panel A) aging augmented circulating leptin significantly (Table 2). A significant interaction "age×time" was found in the factorial ANOVA (Table 2), i.e. in young rats time of day changes were significant, with lowest values at the middle of the light period and higher values at night, whereas time of day changes did not attain significance in old rats. When plasma leptin values were expressed on 100 g body weight, the age-related differences were no longer significant while the daily pattern of plasma leptin found in young rats persisted (Fig. 2, panel B).

As shown in Fig. 3 plasma and adrenal corticosterone levels correlated significantly with plasma ACTH in young rats only. Similarly, plasma leptin values expressed either as total values or on body weight basis, correlated directly with plasma corticosterone only

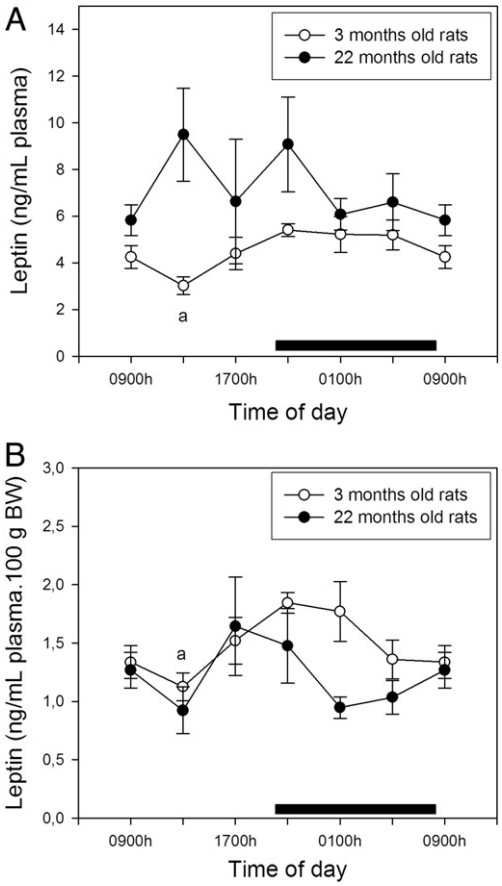

Fig. 2. Twenty-four-hour changes in plasma leptin in young (3 months old) and old (22 months old) rats. Groups of 8–10 rats were killed by decapitation at 6 different time intervals throughout a 24-hour cycle. Bars indicate scotophase duration. Shown are the means±SEM of total leptin concentration (panel A) or leptin concentration expressed on 100 g body weight basis (panel B). Letters indicate the existence of significant differences between time points within each age group after a one-way ANOVA followed by a Student–Newman–Keuls multiple comparisons test, $^{a}p<0.05$ vs. 2100 h. For further statistical analysis, see text.

Table 2
Summary of factorial ANOVA for data of Fig. 2

Source	*df*	Leptin (ng/mL plasma)		Leptin (ng/mL plasma·100 g BW)	
		F	Significance	*F*	Significance
Age	1	8.11	0.036	4.47	0.088
Time of day	5	0.68	0.658	2.19	0.205
Age×time	5	15.1	<0.001	1.16	0.334
Error	10				
	1				
		Mean values±SEM			
Young rats		4.59±0.24		1.22±0.11	
Old rats		7.21±0.69		1.43±0.21	

in young rats (Fig. 4). For the correlation study the number of animals depended on the availability of the simultaneous determination of the hormones examined. We had unexpected technical difficulties in measuring ACTH in young rats and, the simultaneous measurements (ACTH, plasma corticosterone) being available in 23 animals only. To achieve the desired *n* for the results described in Fig. 1, a complementary experiment on daily variation of plasma ACTH had to be performed.

Significant correlations between adrenal and plasma levels of corticosterone were found in both groups (r^2=0.184, F=7.42, p<0.01, and r^2=0.123, F=6.19, p<0.02 for young and old rats, respectively, results not shown).

Discussion

The foregoing results indicate that although aging augmented ACTH levels, the secretion of corticosterone remained low and showed only a brief peak at early scotophase, differing from the sustained high levels found in young rats throughout the night. The data unmasked an early fall in plasma corticosterone during the dark phase in aged rats, a finding which has not been reported previously. Another effect of aging found herein was that the feedback relations between ACTH and plasma or adrenal corticosterone became disrupted. Plasma and adrenal corticosterone levels correlated significantly with plasma ACTH in young rats only. In addition, the significant direct correlation between 24-hour plasma corticosterone and leptin concentration found in young animals was no longer observed in old rats. Therefore, the present results indicate that the normal relations between ACTH and corticosterone, or between corticosterone and circulating leptin, become severely disrupted in aged rats.

Previous studies have often shown activation of the HPA axis or enhanced basal corticosterone levels in aged rats of different strains (Sapolsky et al., 1986a; Sapolsky et al., 1986b; Brodish and Odio, 1989; Dellwo and Beauchene, 1990; Issa et al., 1990; Hauger et al., 1994; Seckl and Olsson, 1995; Sapolsky, 1999; Lucassen and De Kloet, 2001). Indeed, in the present study, plasma corticosterone concentration was increased by approximately 2-fold in aged rats at 1300 h, a time of blood collection in many previous studies. In addition, other reports in the literature failed to find elevated basal corticosterone levels or enhanced stress responses in old animals (Sonntag et al., 1987; Issa et al., 1990; Scaccianoce et al., 1990; van Eekelen et al., 1991; van Eekelen et al., 1992; Morano et al., 1994; Seckl and Olsson, 1995; Cizza

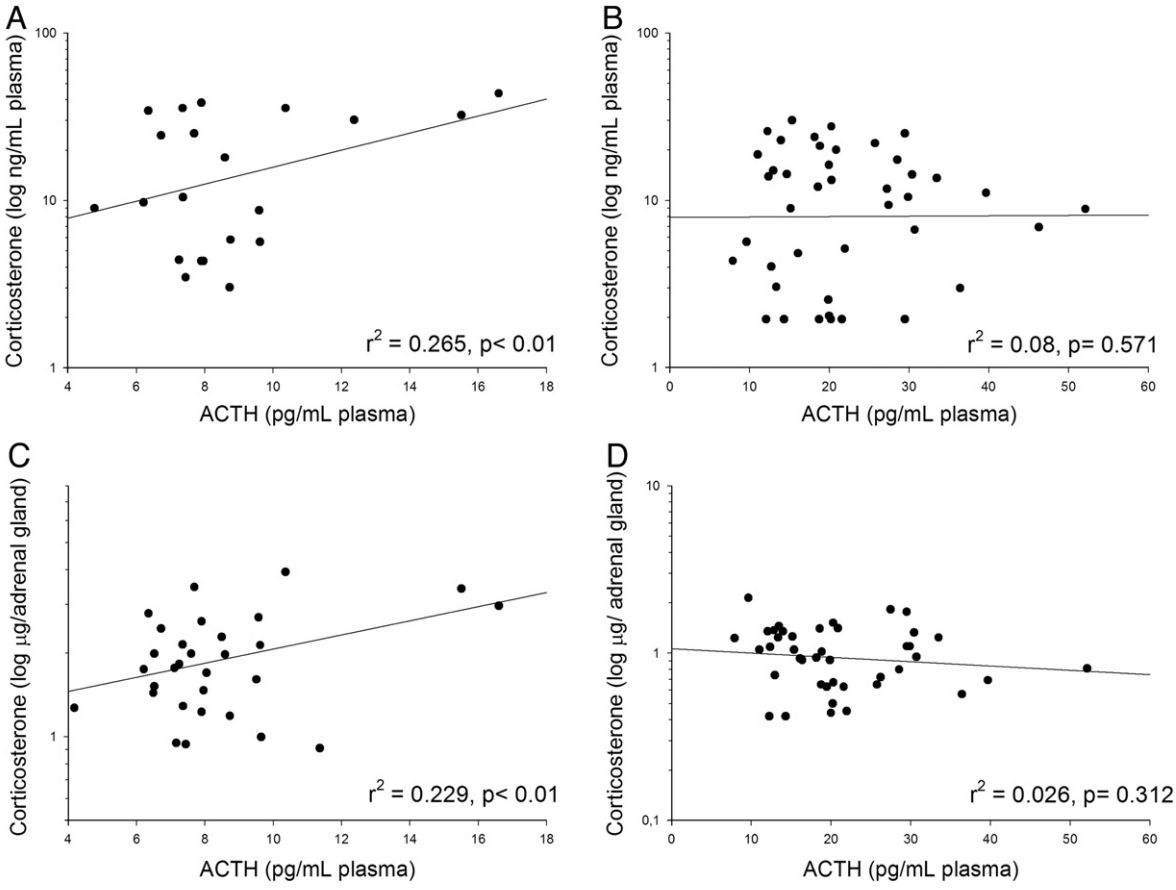

Fig. 3. Semilog scatter diagrams of plasma (upper panels) and adrenal gland corticosterone levels (lower panels) plotted against plasma ACTH concentration. Panels A and C depicted data in 3-month-old rats while panels B and D depicted data in 22-month-old rats. Only in young rats r^2 values were significant (F=9.01 and 9.44 for plasma and adrenal gland corticosterone plots, respectively).

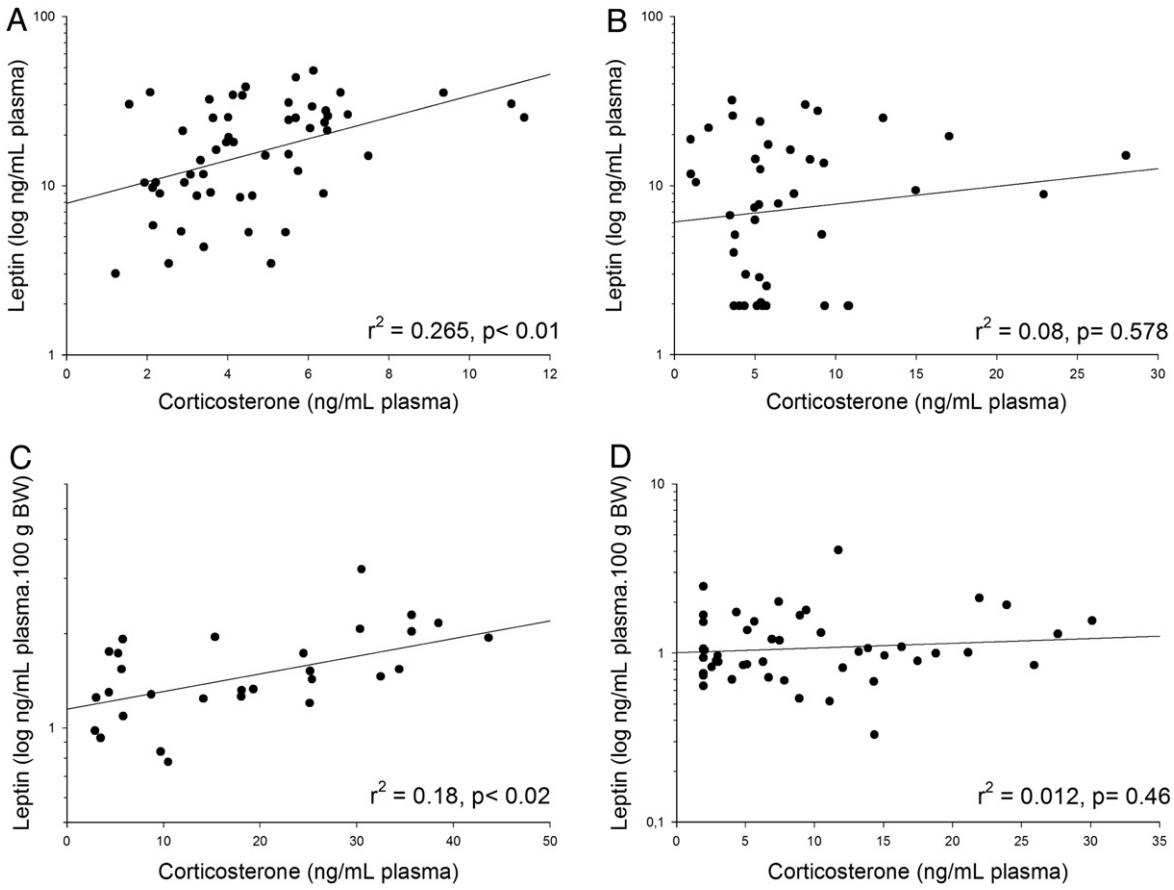

Fig. 4. Semilog scatter diagrams of plasma leptin levels plotted against plasma corticosterone concentration in 3-month-old rats (panel A) and 22-month-old rats (panel B). Only in young rats r^2 values were significant (F=10.8 and 7.42 for total or relative plasma leptin plots, respectively).

et al., 1995; Scaccianoce et al., 1995; Lucassen and De Kloet, 2001; Heine et al., 2004). It seems that HPA axis activation in rodent aging apparently occurs in many, but not all rat strains.

For the correlation study the number of animals depended on the availability of the simultaneous determination of the hormones examined. Due to technical reasons the simultaneous measurement of ACTH and plasma corticosterone was available in a smaller number of young rats than old rats. In any event it must be stressed that the major observation of the present study is not that the very well known fact that plasma ACTH and corticosterone correlate in young rats but that the correlation between both hormones is lost in old animals. The dissociation between enhanced ACTH and reduced glucocorticoid levels appears to be independent of any change in the adrenal expression of ACTH-receptor (ACTH-R) mRNA with advancing age (Han et al., 2001). However, no information is available regarding age-dependent modifications of intracellular mechanisms downstream ACTH-R. Although we have not looked for aging-induced changes in adrenal gland zona fasciculata (ZF) morphology/function, it has been reported ZF hypertrophy in 24-month-old rats (Rebuffat et al., 1992). This hypertrophy occurs at expenses of both volume and number of parenchymal cells, accompanied by increased mitochondrial volume (Markowska et al., 1994). Presumably, morphological changes, due to adrenal exposure to high circulating ACTH levels, represent a ZF compensatory activity for an age-dependent impaired glucocorticoid production.

Leptin is an important peripheral hormonal signal in the regulation of energy homeostasis (Yang and Barouch, 2007; Myers et al., 2008; Vickers, 2007; Bluher and Mantzoros, 2007). Leptin modulates appetite and energy expenditure through an action on the hypothalamic appetite regulating network and the sympathetic nervous system. A major source of circulating leptin is the white adipose tissue but more recent studies have identified several non-adipocyte tissues, including the brain, as capable of producing leptin (Yang and Barouch, 2007; Myers et al., 2008; Vickers, 2007; Bluher and Mantzoros, 2007). Thus, it is possible that circulating leptin levels deriving from varied sources represent a cumulative leptin feedback signal regulating body weight. In rats, blood leptin concentration displays circadian rhythmicity (Xu et al., 1999; Chacon et al., 2005; Perelló et al., 2006).

Leptin secretion is primarily related to body adipose tissue size; leptin gene expression and fasting plasma leptin concentrations are positively correlated with the percentage of body fat (Schwartz et al., 2000). In the present study, the age-associated increase in leptin appeared to be partly due to the fact that the old rats weighed about twice as much as the young rats. Another type of leptin regulation is unrelated to body weight and fat. Plasma leptin levels decline precipitously within 24 h after food deprivation, while feeding stimulates leptin secretion within a few hours (Schwartz et al., 2000).

Since glucocorticoids are a key signal modulating adipocyte function (Shi et al., 2000), the loss of correlation between 24-hour plasma corticosterone and leptin concentrations found in old rats could be directly related to the alteration in adrenal gland function observed at this age. In fact, concomitant increases in circulating corticosterone and leptin levels after different stress stimuli have been described in young rodents (Chautard et al., 1999; Giovambattista et al., 2000). Moreover, it had been earlier reported that bilateral adrenalectomy blunts plasma circulating levels of leptin in rats (Spinedi and Gaillard, 1998). Interestingly, either dexamethasone treatment (Spinedi and Gaillard, 1998) or corticosterone replacement therapy (Chautard et al., 1999) in adrenalectomized rats was effective to enhance circulating leptin levels. In young rats eating ad libitum or

in a restricted manner, a rise in circulating glucocorticoid concentrations preceded both the feeding phase and the rise in leptin secretion following food intake (Xu et al., 1999). The association between the daily patterns of food intake and circulating leptin levels found in young rats became lost with advanced age (Pu et al., 2000). Altogether, previous and present observations argue in favor of a positive interaction between glucocorticoids and leptin in rats, which becomes disrupted in aged animals concomitantly with an overall corticoadrenal dysfunction.

Old rats also demonstrate resistance to the metabolic effects of leptin (Muzumdar et al., 2006). In spite of the higher leptin levels, old rats continue to gain weight, showing resistance to the effect of leptin on food intake and energy expenditure. This resistance persists even after caloric restriction, suggesting that old age per se, independent of age-associated obesity, leads to leptin resistance (Muzumdar et al., 2006).

Challenging individuals to different (physical/psychological) stressors result in increased plasma glucocorticoid levels (Sachser, 1987). We previously reported (Perelló et al., 2006) that social isolation of rats caused a mild stress with unchanged circulating ACTH concentrations and increases in plasma corticosterone to a concentration (about 35 μg/dL) that was however smaller than those reported after acute stress conditions, e.g. restrain (Akana and Dallman, 1997) or bacterial lipopolysaccharide injection (Giovambattista et al., 2000) (more than 70–80 μg/dL). It is of interest that resembling aging, the significant correlations between plasma ACTH and corticosterone, or between corticosterone and leptin, found in control rats, were no longer observed in rats subjected to isolation stress.

It must be noted that circulating ACTH and corticosterone levels reported in the present study were higher during the whole 24-hour period than those measured in single-housed animals after either decapitation (Perello et al., 2003) or i.v. bled (Spinedi and Gaillard, 1998). This was presumably due to the fact that blood samples were collected after decapitation, a procedure known to produce acute stress responsiveness in rats particularly when kept in groups.

Temporal organization is an important feature of the biological systems and its main function is to facilitate adaptation of the organism to the environment (Hastings et al., 2003; Laposky et al., 2008). Aging is capable of perturbing this temporal organization by affecting the shape and amplitude of a rhythm or by modifying the intrinsic oscillatory mechanism itself. Further experiments are needed to assess whether the changes in amplitude as well in timing of 24-hour rhythms of stress-related hormones reported herein in aged rats can be attributed either to an effect on the endogenous clock that modulates the circadian variation of hormone release or to a masking effect on some output(s) of the clock.

There are a number of limitations that must be considered in analyzing the results of this study. Since the present observations are purely descriptive, they do not allow conclusions on either mechanistic insight or cause–effect relationships. For example, although significant correlations between specific measurements made in young, but not aged rats, are reported, the observations do not show causality. It is possible that a common mechanism influencing 24-hour secretion of hormones is affected by age, as opposed to interrelationships between the hormones. One such possibility is the cellular circadian clocks, which are believed to control release of hormones from multiple tissues, including the adrenal gland, adipose, and pancreas. To what extent the age-related changes in cell autonomous circadian clocks are a possible explanation for the observations in the present study deserves to be further explored.

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References

Akana, S.F., Dallman, M.F., 1997. Chronic cold in adrenalectomized, corticosterone (B)-treated rats: facilitated corticotropin responses to acute restraint emerge as B increases. Endocrinology 138 (8), 3249–3258.

Bluher, S., Mantzoros, C.S., 2007. Leptin in reproduction. Current Opinion in Endocrinology, Diabetes, and Obesity 14 (6), 458–464.

Born, J., Ditschuneit, I., Schreiber, M., Dodt, C., Fehm, H.L., 1995. Effects of age and gender on pituitary-adrenocortical responsiveness in humans. European Journal of Endocrinology 132 (6), 705–711.

Brodish, A., Odio, M., 1989. Age-dependent effects of chronic stress on ACTH and corticosterone responses to an acute novel stress. Neuroendocrinology 49 (5), 496–501.

Chacon, F., Esquifino, A.I., Perelló, M., Cardinali, D.P., Spinedi, E., Alvarez, M.P., 2005. 24-Hour changes in ACTH, corticosterone, growth hormone and leptin levels in young male rats subjected to calorie restriction. Chronobiology International 22, 253–265.

Chautard, T., Spinedi, E., Voirol, M., Pralong, F.P., Gaillard, R.C., 1999. Role of glucocorticoids in the response of the hypothalamo-corticotrope, immune and adipose systems to repeated endotoxin administration. Neuroendocrinology 69 (5), 360–369.

Cizza, G., Gold, P.W., Chrousos, G.P., 1995. Aging is associated in the 344/N Fischer rat with decreased stress responsivity of central and peripheral catecholaminergic systems and impairment of the hypothalamic–pituitary–adrenal axis. Annals of the New York Academy of Sciences 771, 491–511.

Dellwo, M., Beauchene, R.E., 1990. The effect of exercise, diet restriction, and aging on the pituitary–adrenal axis in the rat. Experimental Gerontology 25 (6), 553–562.

Drafta, D., Schindler, A.E., Stroe, E., Neacsu, E., 1982. Age-related changes of plasma steroids in normal adult males. Journal of Steroid Biochemistry 17 (6), 683–687.

Esquifino, A.I., Castrillon, P., Garcia Bonacho, M., Vara, E., Cardinali, D.P., 1999. Effect of melatonin treatment on 24-hour rhythms of serum ACTH, growth hormone, prolactin, luteinizing hormone and insulin in rats injected with Freund's adjuvant. Journal of Pineal Research 27 (1), 15–23.

Giovambattista, A., Chisari, A.N., Corro, L., Gaillard, R.C., Spinedi, E., 2000. Metabolic, neuroendocrine and immune functions in basal conditions and during the acute-phase response to endotoxic shock in undernourished rats. Neuroimmunomodulation 7 (2), 92–98.

Gotthardt, U., Schweiger, U., Fahrenberg, J., Lauer, C.J., Holsboer, F., Heuser, I., 1995. Cortisol, ACTH, and cardiovascular response to a cognitive challenge paradigm in aging and depression. American Journal of Physiology 268 (4 Pt 2), R865–R873.

Halbreich, U., Asnis, G.M., Zumoff, B., Nathan, R.S., Shindledecker, R., 1984. Effect of age and sex on cortisol secretion in depressives and normals. Psychiatry Research 13 (3), 221–229.

Han, E.S., Evans, T.R., Shu, J.H., Lee, S., Nelson, J.F., 2001. Food restriction enhances endogenous and corticotropin-induced plasma elevations of free but not total corticosterone throughout life in rats. The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences 56 (9), B391–B397.

Hastings, M.H., Reddy, A.B., Maywood, E.S., 2003. A clockwork web: circadian timing in brain and periphery, in health and disease. Nature Reviews Neuroscience 4 (8), 649–661.

Hatzinger, M., Reul, J.M., Landgraf, R., Holsboer, F., Neumann, I., 1996. Combined dexamethasone/CRH test in rats: hypothalamo-pituitary-adrenocortical system alterations in aging. Neuroendocrinology 64 (5), 349–356.

Hauger, R.L., Thrivikraman, K.V., Plotsky, P.M., 1994. Age-related alterations of hypothalamic–pituitary–adrenal axis function in male Fischer 344 rats. Endocrinology 134 (3), 1528–1536.

Heine, V.M., Maslam, S., Joels, M., Lucassen, P.J., 2004. Prominent decline of newborn cell proliferation, differentiation, and apoptosis in the aging dentate gyrus, in absence of an age-related hypothalamus–pituitary–adrenal axis activation. Neurobiology of Aging 25 (3), 361–375.

Issa, A.M., Rowe, W., Gauthier, S., Meaney, M.J., 1990. Hypothalamic–pituitary–adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. Journal of Neuroscience 10 (10), 3247–3254.

Lakatua, D.J., Nicolau, G.Y., Bogdan, C., Petrescu, E., Sackett-Lundeen, L.L., Irvine, P.W., Haus, E., 1984. Circadian endocrine time structure in humans above 80 years of age. Journal of Gerontology 39 (6), 648–654.

Laposky, A.D., Bass, J., Kohsaka, A., Turek, F.W., 2008. Sleep and circadian rhythms: key components in the regulation of energy metabolism. FEBS Letters 582 (1), 142–151.

Lucassen, P.J., De Kloet, E.R., 2001. Glucocorticoids and the aging brain; cause or consequence? In: Hof, P., Mobbs, C. (Eds.), Functional Neurobiology of Aging. Academic Press, New York, pp. 883–905.

Lupien, S.J., de Leon, M., de Santi, S., Convit, A., Tarshish, C., Nair, N.P., Thakur, M., McEwen, B.S., Hauger, R.L., Meaney, M.J., 1998. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. Nature Neuroscience 1 (1), 69–73.

Maes, M., Calabrese, J., Lee, M., Meltzer, H.Y., 1994. Effects of age on spontaneous cortisolaemia of normal volunteers and depressed patients. Psychoneuroendocrinology 19 (1), 79–84.

Markowska, A., Rebuffat, P., Gottardo, G., Mazzochi, G., Nussdorfer, G.G., 1994. Age-dependent changes in the function and morphology of mitochondria of rat adrenal zona fasciculata. Histology and Histopathology 9 (2), 263–268.

Morano, M.I., Vazquez, D.M., Akil, H., 1994. The role of the hippocampal mineralocorticoid and glucocorticoid receptors in the hypothalamo-pituitary-adrenal axis of the aged Fisher rat. Molecular and Cellular Neurosciences 5 (5), 400–412.
Muzumdar, R.H., Ma, X., Yang, X., Atzmon, G., Barzilai, N., 2006. Central resistance to the inhibitory effects of leptin on stimulated insulin secretion with aging. Neurobiology of Aging 27 (9), 1308–1314.
Myers, M.G., Cowley, M.A., Munzberg, H., 2008. Mechanisms of leptin action and leptin resistance. Annual Reviews of Physiology 70, 537–556.
O'Brien, J.T., Schweitzer, I., Ames, D., Tuckwell, V., Mastwyk, M., 1994. Cortisol suppression by dexamethasone in the healthy elderly: effects of age, dexamethasone levels, and cognitive function. Biological Psychiatry 36 (6), 389–394.
Perelló, M., Chacon, F., Cardinali, D.P., Esquifino, A.I., Spinedi, E., 2006. Effect of social isolation on 24-hour pattern of stress hormones and leptin in rats. Life Sciences 78 (16), 1857–1862.
Perelló, M., Gaillard, R.C., Chisari, A., Spinedi, E., 2003. Adrenal enucleation in MSG-damaged hyperleptinemic male rats transiently restores adrenal sensitivity to leptin. Neuroendocrinology 78 (3), 176–184.
Pfohl, B., Sherman, B., Schlechte, J., Stone, R., 1985. Pituitary–adrenal axis rhythm disturbances in psychiatric depression. Archives of General Psychiatry 42 (9), 897–903.
Pu, S., Dube, M.G., Kalra, P.S., Kalra, S.P., 2000. Regulation of leptin secretion: effects of aging on daily patterns of serum leptin and food consumption. Regulatory Peptides 92 (1–3), 107–111.
Rebuffat, P., Belloni, A.S., Rocco, S., Andreis, P.G., Neri, G., Malendowicz, L.K., Gottardo, G., Mazzocchi, G., Nussdorfer, G.G., 1992. The effects of ageing on the morphology and function of the zonae fasciculata and reticularis of the rat adrenal cortex. Cell and Tissue Research 270 (2), 265–272.
Revskoy, S., Redei, E., 2000. Decreased in vitro sensitivity to dexamethasone in corticotropes from middle-age rats. Experimental Gerontology 35 (2), 237–242.
Sachser, N., 1987. Short-term responses of plasma norepinephrine, epinephrine, glucocorticoid and testosterone titers to social and non-social stressors in male guinea pigs of different social status. Physiology and Behavior 39 (1), 11–20.
Sapolsky, R.M., 1999. Glucocorticoids, stress, and their adverse neurological effects: relevance to aging. Experimental Gerontology 34 (6), 721–732.
Sapolsky, R.M., Krey, L.C., McEwen, B.S., 1986a. The adrenocortical axis in the aged rat: impaired sensitivity to both fast and delayed feedback inhibition. Neurobiology of Aging 7 (5), 331–335.
Sapolsky, R.M., Krey, L.C., McEwen, B.S., 1986b. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocrine Reviews 7 (3), 284–301.
Scaccianoce, S., Di Sciullo, A., Angelucci, L., 1990. Age-related changes in hypothalamo-pituitary-adrenocortical axis activity in the rat. In vitro studies. Neuroendocrinology 52 (2), 150–155.
Scaccianoce, S., Nicolai, R., Cigliana, G., Angelucci, L., 1995. Reduced glucocorticoid response to corticotropin secretagogues in the aged Sprague–Dawley rat. Neuroendocrinology 62 (1), 32–38.
Schwartz, M.W., Woods, S.C., Porte Jr., D., Seeley, R.J., Baskin, D.G., 2000. Central nervous system control of food intake. Nature 404 (6778), 661–671.
Seckl, J.R., Olsson, T., 1995. Glucocorticoid hypersecretion and the age-impaired hippocampus: cause or effect? Journal of Endocrinology 145 (2), 201–211.
Seeman, T.E., Robbins, R.J., 1994. Aging and hypothalamic–pituitary–adrenal response to challenge in humans. Endocrine Reviews 15 (2), 233–260.
Sherman, B., Wysham, C., Pfohl, B., 1985. Age-related changes in the circadian rhythm of plasma cortisol in man. Journal of Clinical Endocrinology and Metabolism 61 (3), 439–443.
Shi, X.M., Blair, H.C., Yang, X., McDonald, J.M., Cao, X., 2000. Tandem repeat of C/EBP binding sites mediates PPARgamma2 gene transcription in glucocorticoid-induced adipocyte differentiation. Journal of Cellular Biochemistry 76 (3), 518–527.
Sonntag, W.E., Goliszek, A.G., Brodish, A., Eldridge, J.C., 1987. Diminished diurnal secretion of adrenocorticotropin (ACTH), but not corticosterone, in old male rats: possible relation to increased adrenal sensitivity to ACTH in vivo. Endocrinology 120 (6), 2308–2315.
Spinedi, E., Gaillard, R.C., 1998. A regulatory loop between the hypothalamo-pituitary-adrenal (HPA) axis and circulating leptin: a physiological role of ACTH. Endocrinology 139 (9), 4016–4020.
Spinedi, E., Giacomini, M., Jacquier, M.C., Gaillard, R.C., 1991. Changes in the hypothalamo-corticotrope axis after bilateral adrenalectomy: evidence for a median eminence site of glucocorticoid action. Neuroendocrinology 53 (2), 160–170.
van Eekelen, J.A., Rots, N.Y., Sutanto, W., De Kloet, E.R., 1992. The effect of aging on stress responsiveness and central corticosteroid receptors in the brown Norway rat. Neurobiology of Aging 13 (1), 159–170.
van Eekelen, J.A., Rots, N.Y., Sutanto, W., Oitzl, M.S., De Kloet, E.R., 1991. Brain corticosteroid receptor gene expression and neuroendocrine dynamics during aging. Journal of Steroid Biochemistry and Molecular Biology 40 (4–6), 679–683.
Vickers, M.H., 2007. Developmental programming and adult obesity: the role of leptin. Current Opinion in Endocrinology, Diabetes, and Obesity 14 (1), 17–22.
Waltman, C., Blackman, M.R., Chrousos, G.P., Riemann, C., Harman, S.M., 1991. Spontaneous and glucocorticoid-inhibited adrenocorticotropic hormone and cortisol secretion are similar in healthy young and old men. Journal of Clinical Endocrinology and Metabolism 73 (3), 495–502.
Wexler, B., 1976. Comparative aspects of hyperadrenocorticism and aging. In: Everit, A.V., Burgess, J.A. (Eds.), Hypothalamus, Pituitary, and Aging. Charles C. Thomas Publisher, Springfield, IL, pp. 333–361.
Xiao, C., Sartin, J., Mulchahey, J.J., Segar, T., Sheriff, S., Herman, J.P., Kasckow, J.W., 2006. Aging associated changes in amygdalar corticotropin-releasing hormone (CRH) and CRH-binding protein in Fischer 344 rats. Brain Research 1073–1074, 325–331.
Xu, B., Kalra, P.S., Farmerie, W.G., Kalra, S.P., 1999. Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: Effects of food restriction. Endocrinology 140 (6), 2868–2875.
Yang, R., Barouch, L.A., 2007. Leptin signaling and obesity: cardiovascular consequences. Circulation Research 101 (6), 545–559.