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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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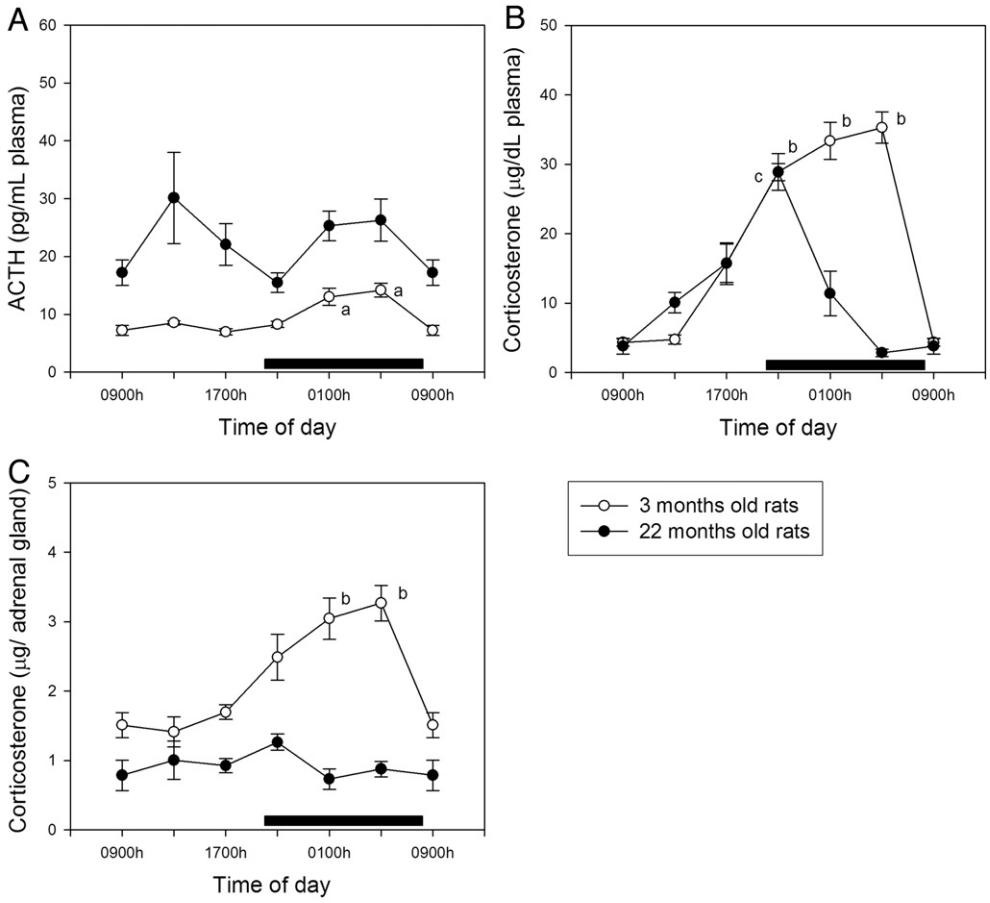

Fig. 1. Twenty-four-hour changes in plasma ACTH (panel A), plasma corticosterone (panel B) and adrenal gland corticosterone (panel C) in young (3 months old) and old (22 months old) rats. Groups of 8–10 rats were killed by decapitation at 6 different time intervals throughout a 24-hour cycle. Bars indicate 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	