The impairment of homeostatic mechanisms in ageing becomes often apparent upon physiological or pathological stimulation. We have previously shown that fasting and refeeding revealed the existence of age-related changes of carbohydrate and lipid metabolism. Because fuel metabolism is partially controlled by corticosteroids we decided to determine the effects of refeeding on adrenal gland morphometry, ACTH, and corticosterone serum levels in young (5 mo) and (20 mo) old male Wistar rats. Fasting for 48 h did not change serum ACTH and corticosterone in both age groups. ACTH level did not change after 24 h of refeeding in young and old rats. However, in old, but not young animals, refeeding resulted in the decrease of corticosterone serum concentration. The relative weight of adrenal gland (% of body weight) did not change significantly with age (p=0.05). Fasting for 48 h induced in old rats but not in young ones increase of relative adrenal weight, and the volume of the reticular zone. Refeeding reduced adrenal volume, fascicular zone and reticular zone. Refeeding for 24 h decreased the total volume of adrenal gland of old rats due to a decline of the volumes of fascicular and reticular zones. In young rats refeeding reduced the volume of reticular zone. It is concluded that refeeding revealed ageing-dependent decline in the secretion of corticosterone, the key hormone of prolonged stress response.

Key words: ageing, fasting, refeeding, ACTH, corticosterone, adrenal gland morphometry

INTRODUCTION

The deterioration of homeostatic mechanisms in aging becomes often apparent upon physiological or pathological stimulation (1). Alterations in the nutritional state, whether short-term or chronic, profoundly affect various
aspects of metabolic and hormonal homeostasis. Fasting and subsequent refeeding belong to nutritional manipulations which reflect naturally occurring periodicity in nutrient supply. Fasting evokes dramatic changes in carbohydrate, lipid, and protein metabolism that are reflected by altered serum levels of key metabolites and hormones (2, 3). We have previously shown that the resynthesis of liver glycogen after fasting was delayed in old rats as compared to adult ones (4). Glucocorticosteroids show permissive effects glucose and fuel metabolism, however, the effects of refeeding on corticosterone and ACTH level in old rats are not known. Therefore we decided to challenge the function and structure of the adrenal gland of young and old rats in the experimental setting of fasting/refeeding. The aim of our study was to determine the effects of fasting and refeeding on the serum concentrations of ACTH and corticosterone, and on the size of adrenal glands and its main components of young mature and old rats.

MATERIAL AND METHODS

Animals

Inbred male Wistar rats aged 5 months (young, 381±21.5 g, n=16, mean±SD), and 24 months (465±36.3g, n=19) were used. The mean and the maximal life span of this rat colony was 27 and 36 months, respectively (4). The animals were housed 3 per cage and maintained on a controlled light schedule (light on 7:00-19:00) at 20±1°C. They were fed a standard diet containing (w/w) 13% protein, 55.5% carbohydrate, 2.5% lipid and 29% indigestible compounds, (LSM, Motycz, Poland). The experiments were carried out according to the NIH guide for the care and use of laboratory animals.

Dietary manipulation and sampling

Control rats were fed ad libitum and sampled correspondingly at the same time of the day as fasted and refed animals to exclude possible diurnal variations in hormone levels. Other animals were fasted starting from 8:00 hour and sampled after 48 hours, or fasted for 48 hours, then provided with food at 8:00 (start of refeeding) and sampled 24 hours thereafter. Food consumption was measured for group of 3 rats present in one cage and changes in body weight were determined for each rat. Normally fed control young and old rats consumed over 24 hours 8.9±0.9 g (mean±SE), and 7.9±0.8 g chow per 100 g b.w., respectively. Young rats fasted for 48 hours and refed for 24 hours consumed 10.8±1.7/100g b.w. Old fasted/refed rats consumed 9.2±1.7 g of chow per 100 g b.w. At each time point 5-6 rats per group were bled from the abdominal aorta under thiopental anaesthesia. Blood was collected into ice-chilled tubes, centrifuged at 4°C, and the serum was separated immediately and stored at -30°C until required for analysis.

Morphometry of rat adrenal gland

Rats were perfused with 4 % paraformaldehyde in phosphate buffered saline, adrenal glands were removed and fixed in Bouin's solution, embedded in paraffin and serially cut at 10 µm. Sections were stained with hematoxylin and eosin. Morphometric analysis were made according to the Cavalieri method. Every 10th section was analysed under the final magnification of 100x by the use
of semiquantitative program Lucia 3.1. except for the region where the the glomerular zone and the core of the adrenal gland were easily identifiable at the sections. However, in some regions, where there was no difficulty in identifying areas where fascicular zone merged with reticular zone, the border between them was assumed arbitrarily based. The circumference of each zone was was measured by the light pen, and the values were stored in a spreadsheet before further analyses.

Hormone analysis

Hormone concentrations were measured with commercially available radioimmunoassays for rat ACTH and rat corticosterone (DRG, USA).

Statistical analysis

Results are expressed as the mean±SE. Analysis of variance (ANOVA) was used for comparison of mean values, and the Neuman-Keuls test was used for group analysis (Statistica PL5.0, Systat, Krakow, Poland). A normal distribution had been previously observed. The minimum level of significance was set at P<0.05.

RESULTS

The effect of fasting and refeeding on the body mass of rats

Young fed rats were significantly smaller than old ones (381±22 g, n=16 vs 465±36 g, n=19, p<0.001). Fasting for 48 hours decreased body mass of young rats by 13.0% (from 382±24 to 331±23 g, n=11) and body mass of old animals by 12.4% (from 466±35 to 409±38 g, n=14).

Refeeding of young rats that were fasted for 48 h increased body mass by 9.9% (345±23 vs 379±22 g, n=5). Refeeding of old rats previously fasted for 48 h increased body mass by 6.8% (fasted 404±38 g, refed 432±39 g, n=9). The difference in the increase of body mass of refed young and old rats was statistically significant (p<0.01).

Morphometry of the adrenal gland

The mass of adrenal glands was significantly higher in old as compared to young rats. However, the relative mass of adrenal gland (% of body mass) did not change with age (Table 1, p=0.05). There was a tendency to a higher volume of adrenal glands in normally fed (control) old rats as compared to young ones, however, the difference was not statistically significant (Table 2, p=0.06).

Fasting for 48 hours to old rats but not to young ones caused an increase of adrenal mass as well as relative adrenal mass (Table 1), and enhanced the volume of the reticular zone (Table 2).

Refeeding for 24 hours decreased the total volume of adrenal glands in both age groups of rats (Table 2). The decrease was caused by a significant decline of fascicular and reticular zone volumes in the adrenals of old rats, and a decline in the volume of reticular zone of young animals (Table 2).
ACTH and corticosterone concentrations in the blood serum of fasted and refeed rats

Serum ACTH concentrations were not different in control young and old rats (Fig. 1). In both age groups fasting and refeeding did not change serum ACTH level as compared to that of fed animals.
Fig. 1. The effect of fasting and refeeding on serum ACTH concentration. Empty bars, fed rats (controls), hatched bars, rats fasted for 48 h, filled bars, rats fasted for 48 h and then refed for 24 h (refed). Values are means ± SEM, n=5-7 for each group.

Fig. 2. The effects of fasting and refeeding on serum corticosterone concentration. Empty bars, fed rats (controls), hatched bars, rats fasted for 48 h, filled bars, rats fasted for 48 h and then refed for 24 h (refed). Values are means ± SEM, n=5-7 for each group. *& p<0.01 vs old fasted rats.
Serum corticosterone concentration did not differ between young and old control rats. In young animals fasting and refeeding did not change serum corticosterone concentration. In refed young rats, the serum corticosterone level did change significantly. In contrast, in old refed rats serum corticosterone concentration was 5 times lower than in fasted animals (Fig. 2).

DISCUSSION

Kaneda et al. showed that following starvation plasma corticosterone increased significantly in young rats (9 week old) but not in middle aged rats (72 week old). Young rats showed a tendency towards an increase in plasma ACTH, no significant change was observed in middle-aged rats. Adrenal sensitivity of corticosterone output for the increment of plasma ACTH during starvation was lower in middle-aged rats than in young rats (5).

The effects of ageing on adrenal functioning are conflicting. Corticosterone levels in old unstimulated animals when compared to young animals were found either increased, unchanged or decreased after a maximum at an intermediate age (6). Similarly, controversies exist regarding ACTH level in aged rats. The mechanism underlying such changes is still a matter of debate.

Studies in HPA axis suggested increase or decrease hypothalamus activity whereas others emphasised lesser functional ability of the adrenal glands.

The ageing process has profound effect on structure and function in adrenal glands. Maximum serum ACTH induced corticosterone production was found to be lower in old group of rats (7). Adrenal inner zone hypertrophy may be a response capable on compensating for the age-dependent decrease in glucocorticoid secretion activity of fascicular zone and reticular zone cells (8).

Structural data of fascicular zone showed age-related increase in cell volume, decrease in mitochondria and smooth endoplasmic reticulum volumes and increase in lipid droplets and lipofuscin granules volumes. The aged-related decrease in mitochondria and SER volumes is consistent with the decrease of serum corticosterone (6).

Fasting increased corticosterone from 162±25 ng/ml to 340±24 ng/ml and ACTH from 46.7±4.7 pg/ml to 103±10.8 pg/ml. Exogenous leptin in fasting blunted the rise in corticosterone to 238±27 ng/ml and ACTH to 47.3±5.1 pg/ml (9).

Starved rats were marked increase in plasma ACTH and corticosterone during the dark (10, 11). Fasting for 3 days of old aged rats decreased corticosterone serum level to lower value than in young (2 mo old) animals (12).

In ageing rats functional ability of the adrenal gland seems to be suppressed. Ageing impaired refeeding response. Middle-aged rats showed smaller plasma corticosterone response than young rats for 4 days of starvation. Starvation induced increase in neuropeptide Y mRNA and decrease in proopiomelanocortin mRNA were smaller in middle-aged rats than in young rats (5).
Refeeding is associated with energy conservation with reduction in energy expenditure. Bilateral adrenalectomy reduced differences in energy expenditure between refeed group and control and attenuated body fat gain from a three- to two-fold increase above control group (13). The enhanced fat intake was positively correlated with the elevations in corticosterone observed at the start of the refeeding. Adrenalectomy reduced dietary fat intake in refeed rats (14).

We conclude that lower increase body weight in refeeding can be caused by lower level of corticosterone in old rats. Reduced corticosterone level in old rats can be coherent with reduced inner zone in refeeding.

Acknowledgements: This work was supported by the State Committee for Scientific Research Grant ST-12.

REFERENCES


Received: September 15, 2006
Accepted: October 2, 2006

Author’s address: Prof. dr Zbigniew Kmiec, Dept. Histology and Immunology, Medical University of Gdansk, ul. Debinki 1, 80-211 Gdansk, Poland. Phone: (+48 58) 3491437, fax 3491436; e-mail: zkmiec@amedec.amg.gda.pl