Ultrastructural observations on the hypothalamic arcuate nuclei of aged rats in the fasting/refeeding model

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The arcuate nucleus of the hypothalamus (ARH) is involved in the control of energy homeostasis. This is the first study on the ultrastructural response of ARH neurons in aged rats after short-term fasting and subsequent refeeding. Male Wistar rats (24 weeks old) were fasted for 48 or 96 hours and were then refed for 24 hours. The controls were normally fed. The rats received water ad libitum. In both groups of fasting animals, we observed a rearrangement of the arcuate rough endoplasmic reticulum (RER) and Golgi complexes to form membranous whorls. Moreover, refeeding for 24 hours did not reverse this process. The RER was frequently found to be well organized into lamellar bodies composed of several cisternae. The membranous whorls were composed of concentric layers of endoplasmic reticulum and Golgi complexes. In addition, multiform lipofuscin granules were observed in close relationship with Golgi complexes and membranous whorls. Lipofuscin granules within the neurons of the arcuate nucleus are assumed to be a morphological manifestation of oxidative stress phenomena, which are presumably implicated in the formation of membranous whorls in both fasting and fasting/refed animals. This observation correlates with a significant increase in 8-isoprostane serum levels in the fasting and fasting/refed animals as compared to the fed control rats. (Folia Morphol 2009; 68, 2: 79–83)

Key words: arcuate nucleus, fasting/refeeding, whorls, oxidative stress

INTRODUCTION
The arcuate nucleus of the hypothalamus (ARH) plays an important role in the control of food intake and energy homeostasis. Oxidative stress-dependent signal transduction via leptin and the leptin receptor has been reported in a number of cell systems [7, 9]. However, the significance and relevance of oxidative stress in ARH is unclear. Membranous whorls, composed of concentric layers of endoplasmic reticulum and cisternae of Golgi complex, were only found in the ARH of rats exposed to mercuric chloride, being an oxidative stress inducer [8].

Whereas, testosterone deficiency following either castration or chronic morphine treatment [3, 12] stimulated the increase in ARH membranous whorls. Interestingly, testosterone therapy inhibited the process. The purpose of the study was to investigate the effect of fasting and subsequent refeeding on ultrastructural alterations of the endoplasmic reticulum/Golgi network in the arcuate nucleus of aged rats as well as potential correlation of these changes with oxidative stress phenomena. The results may prove useful for a better understanding of obesity, including the process of losing weight, especially in...
the elderly, as well as the pathology of certain en-

doctrine diseases.

MATERIAL AND METHODS

Animals

The study was performed on aged (24-month-
-old) male Wistar rats fasting for either 48 hours
(Group II) or 96 hours (Group III) and then being
referred for 24 hours. The control rats (Group I) were
fed ad libitum. The animals were housed 2 per cage
and they were maintained at 20 ± 1°C on a con-
trolled 12-hour light regime (with the light on from
07:00 to 19:00). The rats were fed with standard
chow containing (w/w) 13% protein, 55.5% carbo-
hydrate, 2.5% lipid, 1% calcium, 0.75% phosphates,
and 27% indigestible compounds (Labofeed B,
Kcynia, Poland). Animals were cared for and treat-
ed according to the “European Convention for the
Protection of Vertebrate Animals Used for Experi-
mental and Other Scientific Purposes”. The study
was approved by the Local Ethical Committee for
Animal Experiments in Gdańsk, Poland.

Dietary manipulation and sampling

The control rats (Group I) were sampled at the
same time as the fasting/refed animals. The rats in
Groups II and III were sacrificed at 8:00 after 24 hours
of feeding. Food consumption was calculated per
2 rats housed together in a cage, and the body weight
change was determined for each rat individually. The
initial body weight of the rats in groups I, II, and III
was 457 ± 3 g, 507 ± 3 g, and 520 ± 10 g, respec-
tively (mean ± SD, n = 4 in each group). The body
weight of the rats in groups II and III after fasting
was 455 ± 5 g and 460 ± 20 g, whereas after fast-
ing/refeeding it was 482 ± 8 g and 477 ± 18 g,
respectively. The rats in group I consumed 9.3 ± 1.3 g
of chow per 100 g bodyweight over 24 hours. After
24 hours of refeeding the average food consump-
tion of the old rats in Groups II and III was 9.5 ± 0.8 g
and 8.5 ± 0.3 g of chow per 100 g bodyweight,
respectively. The rats were provided with water ad
libitum.

Ultrastructural study

The animals, both the control and fasting/refed
groups, were deeply anaesthetized with 10% ket-
amine and then perfused transcardially with a mix-
ture of 4% paraformaldehyde and 1% glutaralde-
hyde in 0.05 M phosphate buffer (pH 7.4) con-
taining CaCl2. After perfusion, the brains were re-
moved from the skulls and stored in the same fix-
ative overnight at 4°C. Next, from the tissue blocks
containing hypothalamus, serial coronal slices
(500 μm thick) were cut on a vibratome 1000S
(Leica, Germany). Small tissue specimens contain-
ing arcuate nucleus were taken bilaterally under a
binocular stereomicroscope and then left in the
fixative for 3 hours and post-fixed in 1% osmium
tetroxide for 2 hours. After dehydration in alco-
hols and propylene oxide, the specimens were em-
bedded in Epon 812. In order to precisely localize
the individual arcuate nuclei, Epon semithin (1 μm)
sections were cut on a Reichert OmU3 ultramicro-
tome, stained with 1% toluidine-blue solution and
examined by light microscopy. After a suitable area
was found, the specimens were trimmed and ul-
trathin sections were then cut. The ultrathin sec-
tions were stained with uranyl acetate and lead
citrate and examined in a JEM 1200 EX II electron
microscope.

Immunochemistry

Moreover, total 8-isoprostane serum levels were
measured using commercially available Elisa kits
(Cayman, Ann arbor, MI, USA), as a reliable marker
of oxidative stress induced lipid peroxidation in vivo.

RESULTS

Group I (fed control)

In general, the neurons of the arcuate nucleus
were round in shape and contained a large, cen-
trally placed nucleus (Fig. 1). The nuclear envelope
exhibited deep invaginations. Usually each neu-
ron contained a prominent nucleolus localized ec-
centrically. A narrow band of cytoplasm was poor
in organelles: sparse cisternae of the rough endo-
plasmic reticulum (RER), patches of Golgi complex-
es in the perinuclear region, and few lipofuscin
granules. Other organelles including ribosomes
and mitochondria were distributed quite evenly
in the cytoplasm. Occasional short lamellar bod-
ies, composed of two parallel cisternae of the RER
with electron dense material in between, were ob-
erved in the cytoplasm. The membranous whors
consisted of closely apposed concentric cisternae
of the RER devoid of ribosomes were found in the
control animals very rarely.

Groups II and III (having fasted for 48 h
or 96 h and then having been refeed for 24 h)

In fasted as well as in fasted/refed animals, the
neurons of the arcuate nucleus were characterized
by large, irregular nuclei with prominent nucleoli
of short independent fragments randomly dispersed throughout the cytoplasm or longer ones anastomosing one another, with a tendency to form lamellar bodies (Figs. 3, 4A, B, 5A) and membranous whorls (Figs. 4A, 5B). Lamellar bodies were formed from two to four parallel cisternae of the RER with electron dense material between them. They became longer than in the control animals and some of them were bent, probably participating in the formation of membranous whorls (Fig. 5A). Membranous whorls, however, were characterized by closely apposed concentric cisternae of the RER devoid of ribosomes. Sometimes dilatations could be seen extending from the ends of the endoplasmic reticulum (Fig. 4A). The outermost layers of the lamellar bodies and membranous whorls were irregularly covered with ribosomes and often appeared to be continuous with the RER cisternae (Figs. 4A, 5A). One to three membranous whorls were observed in a single cross section. Moreover, some very well developed Golgi complexes (Figs. 3, 4B, 5C) displayed the early stages of whorl formation by means of involution of their cisternae. All the membranous whorls usually appeared in close association with the Golgi complexes and membranous whorls.
The serum 8-isoprostane levels in the fasting/refed rats was 12.57 ng/mL, whereas in the fasting only animals it averaged 10.84 ng/mL — in both cases being significantly higher compared to the control level of 5.47 ng/mL (p < 0.05) (Fig. 6). There was no statistically significant difference between the fasting only and fasting/refed groups of animals.

**DISCUSSION**

In our study, in the arcuate neurons of aged control rats, we noted only occasional membra-
nous whorls. Under normal conditions, Brawer [2] described them in the ependymal tanyocytes of the arcuate region in male rats, whereas van Houten and Brawer [13] observed these structures in normal male rats in the ventrolateral part of the hypothalamic ventro-medial nucleus (VMN). Our previous studies [6] did not indicate these bodies because the specimens were taken from the central part of the VMN. The results of our studies, in terms of the response of arcuate neurons to fasting and subsequent refeeding, are consistent with the studies of Kiss [5], who also observed a wide variety of endoplasmic reticulum formations after repeated immobilization stress in the ventromedial nucleus of the hypothalamus of male rats. In addition, we observed the early stages of membranous whorl formation from the Golgi complexes. It was probably too short for expression of typical morphology of whorls. Our studies indicated that refeeding for 24 hours did not inhibit the process of membranous whorl formation. Brawer [1] and Price et al. [12] also observed the appearance of whorls in the arcuate neurons after castration or chronic morphine treatment, which had reduced the testosterone level in the blood of male rats. Interestingly, testosterone replacement therapy inhibited the process. It is possible, as some authors have suggested [1, 3, 4] that whorls in the arcuate nucleus may mark the sites of luteinizing hormone-releasing hormone (LH-RH) synthesis. This phenomenon was confirmed by immuno-electron microscopy [11]. Whorls were observed in ovariectomised rats [4] and in a mercury-treated female hamster [8] that had low levels of ovarian steroids. Thus, it is possible that the whorls are a sign of increased activity of the arcuate neurons due to the loss of feedback control from the gonads [1]. Since whorls are associated with RER and Golgi complex, they may contain carrier proteins and enzymes associated with the release of LH-RH [4] or may be involved in the early stages of the synthesis of LH-RH [10]. In our studies, as well as those of other authors [11], there was an increase of lipofuscin/lysosome granules in the vicinity of the whorls and Golgi region. Some of them may capture LH-RH granules [11]. Lipofuscin granules have been demonstrated to be secondary lysosomes of heterogenous content that derive from autophagic vacuoles. Interestingly, formation of lipofuscin granules is assumed to be a result of intracellular oxidative stress phenomena, and a failure of their removing mechanism during ageing is probably the cause of lipofuscin accumulation in senescent neurons. Lipofuscin itself may in turn be another reason for increased oxidative stress responsible for the formation of whorls. In our experimental model, significant accumulation of lipofuscin in the close proximity of the Golgi complex and membranous whorls strongly suggests that oxidative stress may trigger topological remodelling of those membranes. Moreover, we observed a significant increase (over 2-fold) of the serum level of 8-isoprostan, being a widely accepted marker of in vivo lipid peroxidation, in the groups of fasting and fasting/refed rats as compared to the fed controls. Further research is needed to elucidate the intracellular mechanism of the whorl formation in neurons of ARH during short-term fasting.

REFERENCES