VIA MEDICA

ORIGINAL ARTICLE

Folia Morphol.
Vol. 63, No. 1, pp. 25–35
Copyright © 2004 Via Medica
ISSN 0015–5659
www.fm.viamedica.pl

The effect of fasting and refeeding on the ultrastructure of the hypothalamic paraventricular nucleus in young and old rats

Jolanta Kubasik-Juraniec[1], Zbigniew Kmieć[2], Cecylia Tukaj[1], Teresa Rudzińska-Kisiel[1], Grażyna Kotlarz[2], Leszek Pokrywka[2], Andrzej Myśliwski[2]

[1]*Laboratory of Electron Microscopy, Medical University, Gdańsk, Poland*
[2]*Department of Histology and Immunology, Medical University, Gdańsk, Poland*

[Received 28 August 2003; Revised 6 November 2003; Accepted 6 November 2003]

In order to explore the morphological basis of the altered feeding behaviour of old rats, an ultrastructural investigation of the magnocellular neurons of the hypothalamic paraventricular nucleus (PVN) was performed. Young and old male Wistar rats, 5 and 24 months old, respectively, and with each age group comprising 12 animals, were divided into 3 groups. The rats in Group I were used as controls (normally fed), the rats of Group II were fasted for 48 hours and in Group III the rats were fasted for 48 hours and then refed for 24 hours. The brains were fixed by perfusion and histological and ultrathin sections were obtained by routine methods. Common features of the magnocellular PVN neurons of young and old rats were abundant Golgi complexes and short fragments of RER localised at the cell periphery. In contrast to young rats, the PVN neurons of old animals showed deep indentations of the nuclear envelope and age-related residual bodies. In both age groups fasting for 48 hours led to the expansion of the Golgi complexes and dilatation of RER cisternae. In contrast to those in fed rats, RER cisternae in the neurons of old fasted animals were situated between the nuclear envelope and the Golgi zone. Prolonged RER cisternae were distributed in the peripheral cytoplasm of refed old rats. Our observations suggest that at the ultrastructural level the process of ageing does not change the responsiveness of magnocellular PVN neurons to fasting-refeeding.

Key words: ageing, paraventricular hypothalamic nucleus, electron microscopy, fasting, refeeding, rat

INTRODUCTION

The process of ageing diminishes the capability of organisms to adapt to changes in the internal and external environment. Fasting and subsequent refeeding are nutritional manipulations which reflect a naturally occurring periodicity in nutrient supply. We have previously demonstrated that old rats that were refed after fasting for 48 hours showed significant changes, as compared to young animals, in the rate of liver glycogen resynthesis [13], structure of the thyroid follicles [9], and serum concentrations of iodothyronines, parathormone, and calcitonin [8, 10]. Moreover, during the refeeding experiments we noticed that the food intake of old rats was lower than that of young animals. Hypothalamic nuclei, such as the lateral hypothalamic area (LHA, "hunger centre") and ventromedial hypothalamic nucleus (VMN, "satiety centre"), as well as the arcuate (ARC) and paraven-

Address for correspondence: Jolanta Kubasik-Juraniec, Laboratory of Electron Microscopy, Medical University, ul. Dębinki 1, 80–210 Gdańsk, Poland, tel: +48 58 349 15 00, fax: +48 58 349 14 36, e-mail: kubasik@amg.gda.pl

tricular (PVN) nuclei that receive afferent neural and hormonal signals, were shown to control food intake, energy homeostasis, and body mass [3, 6]. In a previous work we investigated ultrastructural alterations in the VMN neurons of old rats that were fasted for 2 days and then refed for 1 day as compared to the alterations in young ones [11]. Because other hypothalamic centres that play an important role in the integration of neural and hormonal signals involved in the control of food intake may also express age-related alterations, we decided to compare the effects of fasting/refeeding on the ultrastructure of the paraventricular neurons of young and old rats.

MATERIAL AND METHODS

Animals

Inbred male Wistar rats aged 5 months (young, 338 ± 22 g, n = 12, mean ± SD), and 24 months (old, 483 ± 33 g, n = 12) were used. The age groups were divided into 3 subgroups (n = 4 each). The mean and the maximal life span of the rat colony were 26 and 32 months respectively. The details of the animals' maintenance were described in our previous report [11]. The study was approved by the Local Ethical Committee for Animal Experiments, Gdańsk, Poland.

Dietary manipulation and sampling

The control rats (Group I) were fed *ad libitum* and sampled correspondingly at the same time of the day as the fasted and refed animals. Other animals were fasted starting from 8 a.m. and sampled after 48 hours (Group II), or fasted for 48 hours, provided with food at 8 a.m. (start of refeeding) and sampled 24 hours afterwards (Group III). Food consumption was measured for 2 or 3 rats present in one cage and changes in body weight were determined for each rat. The body weight of young rats in Groups I, II and III was 342 ± 27 g (mean ± SD) 302 ± 21 g, and 338 ± 17 g, respectively. The body weight (b.w.) of old rats in Groups I, II and III was 485 ± 43 g, 448 ± 44 g, and 468 ± 37 g, respectively. Young and old rats of Group I consumed 10.8 ± 1.1 g, and 9.3 ± 1.3 g chow per 100 g b.w., respectively, over 24 hours. After 24 hours of refeeding the average food consumption of young and old rats was 13.8 ± 1.1 g and 9.5 ± 0.8 g of chow per 100 g b.w. respectively. The electron microscopic and histological procedures employed in the present study have been recently described in detail [11], and therefore will be outlined only briefly. The rats were anaesthesised with sodium pentobarbital (50 mg/kg b.w., *ip*), and perfusion fixed through transcardial puncture with 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer (pH 7.4) with $CaCl_2$. After perfusion the brains were removed and left overnight in the same fixative. The tissue blocks containing the hypothalamus were dissected out, mounted on vibratome, and 500 μm coronal serial slices were cut. Small tissue specimens containing PVN were taken unilaterally (left side) from the slices, left overnight in fixative and postfixed in 1% OsO_4. After dehydration in alcohols and propylene oxide specimens were embedded in Epon 812. To confirm that the specimens had been taken from the appropriate brain area, the slices from the remaining brain tissue were fixed in 10% formalin for 2 days and embedded in paraffin. Microtome sections (20 μm) were stained with haematoxylin and eosin. Semithin Epon sections (1–2 μm thick) were cut from each block with Reichert Om U3 ultramicrotome, placed on glass slides and stained with 0,1% toluidine blue. The exact localisation of the magnocellular part of PVN (mPVN) was confirmed by comparing under the light microscope semithin sections of the unilateral side with histological sections of the contralateral one. Once the mPVN was identified, the Epon blocks were trimmed to obtain ultrathin sections for electron microscopic studies. The sections were stained with uranyl acetate [18] and lead citrate [16] and examined with a JEM 1200 EX II electron microscope. In total, 604 electron micrographs of the mPVN were chosen for analysis.

RESULTS

The magnocellular part of PVN can be recognised as an aggregation of large neurons localised laterally to the third ventricle [14]. The cell bodies are in direct apposition to one another and separated by neuropil or thin astroglial processes. Occasionally more than one nucleolus was observed in the same cell nucleus (Fig. 1).

Young animals

Group I (fed control). The PVN neurons of the control rats were round or fusiform in shape and contained large nuclei, usually localised in the centre of the cell bodies (Fig. 1). Heterochromatin was randomly distributed with fine aggregations of granular material adjacent to the nuclear envelope, which was generally smooth or mildly invaginated. Prominent nucleoli were usually localised in an eccentric position. The cytoplasm of the perikarya showed well developed rough endoplasmic reticulum (RER), the cisternae of which were dispersed as single discrete sacs or arranged in stacks at the cell periphery. Polyribosomes were uniformly dis-

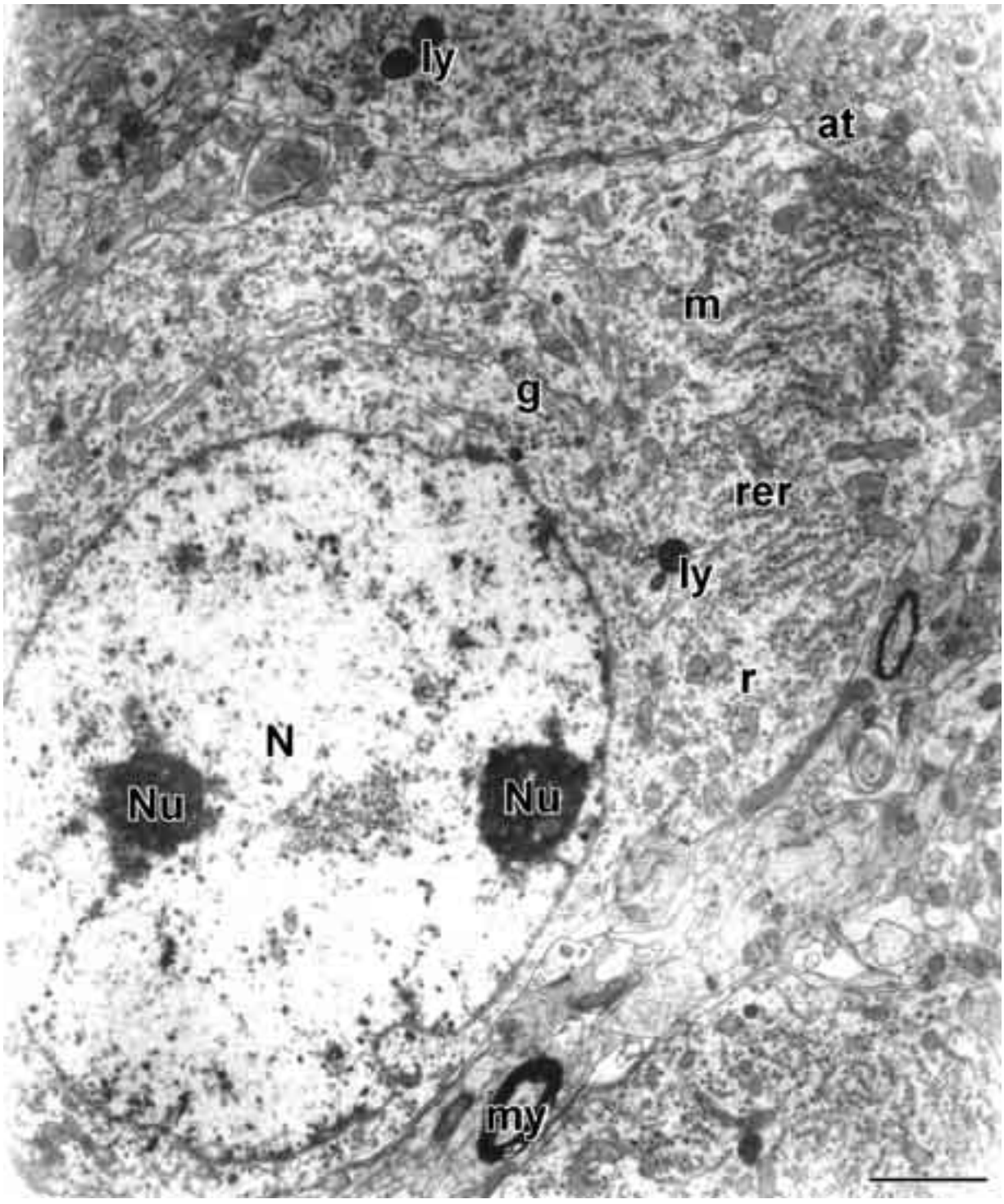


Figure 1. Control (normally fed) young rat. The large nucleus (N) surrounded by slightly invaginated nuclear envelope contains two prominent nucleoli (Nu). Prominent Golgi complexes (g), parallely arranged RER cisternae (rer), polyribosomes (r), mitochondria (m), neurosecretory vesicles (arrow) and occasional lysosome-like bodies (ly) are also present in part of the neighbouring neuron. Axonal terminal (at), myelinated axon (my). Bar: 2 μm.

tributed throughout the cytoplasm. Golgi complexes composed of several layers of flat cisternae were often distributed in the perinuclear area. Clear vesicles and numerous neurosecretory granules were observed in the vicinity of the Golgi structures. The mitochondria, in the form of slender rods or oval bodies, were distributed fairly evenly in the cytoplasm. Single lysosome-like bodies were seen all over the cytoplasm.

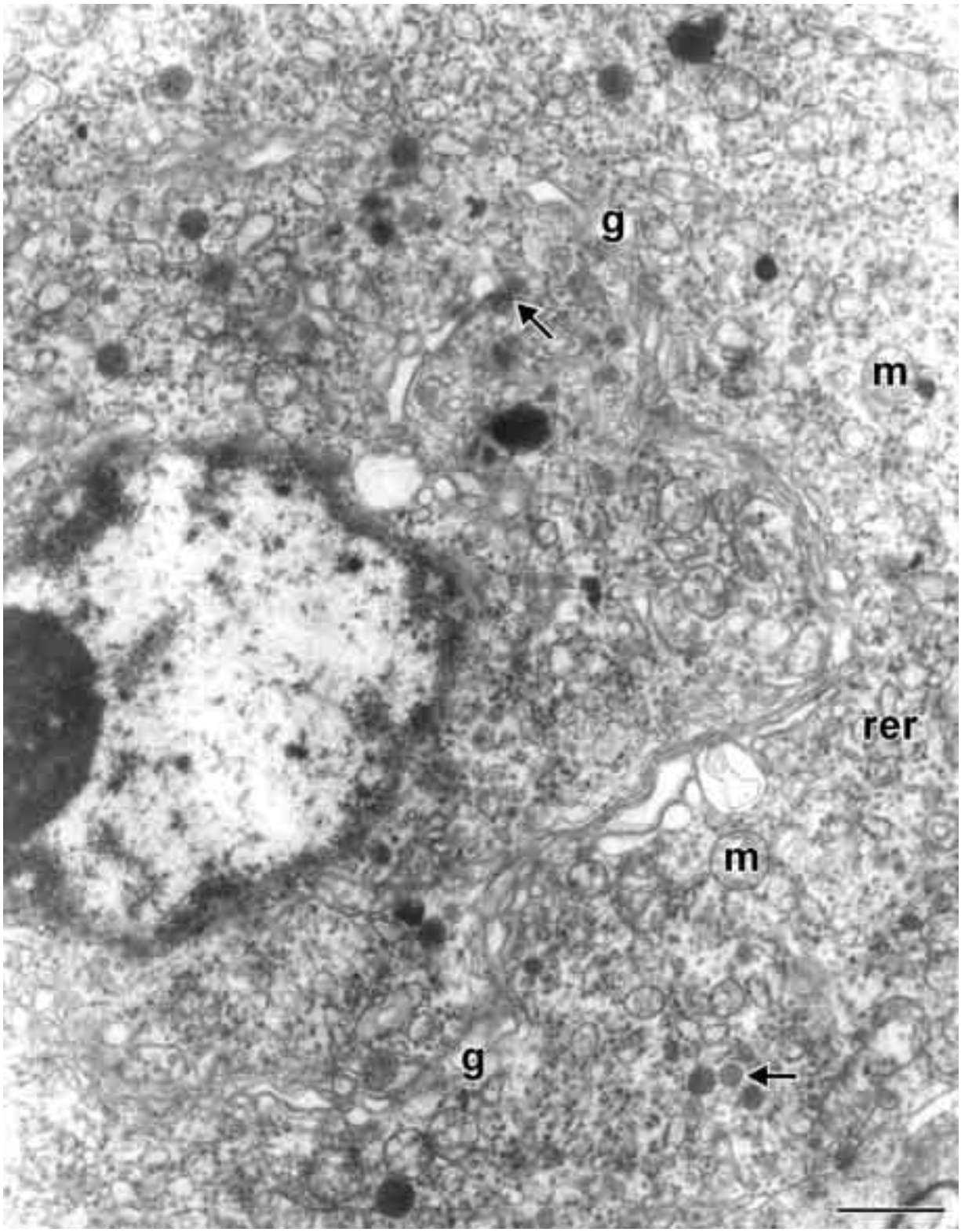


Figure 2. Young rat fasted for 48 h. The extensive multilayered Golgi complexes (g) with numerous small vesicles surround the nucleus. Distended cisternae of fragmented RER (rer) are randomly distributed in the cytoplasm. Neurosecretory granules (arrows); mitochondria (m). Bar: 1 μm.

Group II (fasted for 48 hours). Fasting for 2 days resulted in a prominent expansion and dilatation of Golgi complexes with accompanying small clear vesicles at their periphery (Fig. 2). Neurosecretory granules were localised close to Golgi complexes (Fig. 2). Discreet vacuolisation of mitochondrial

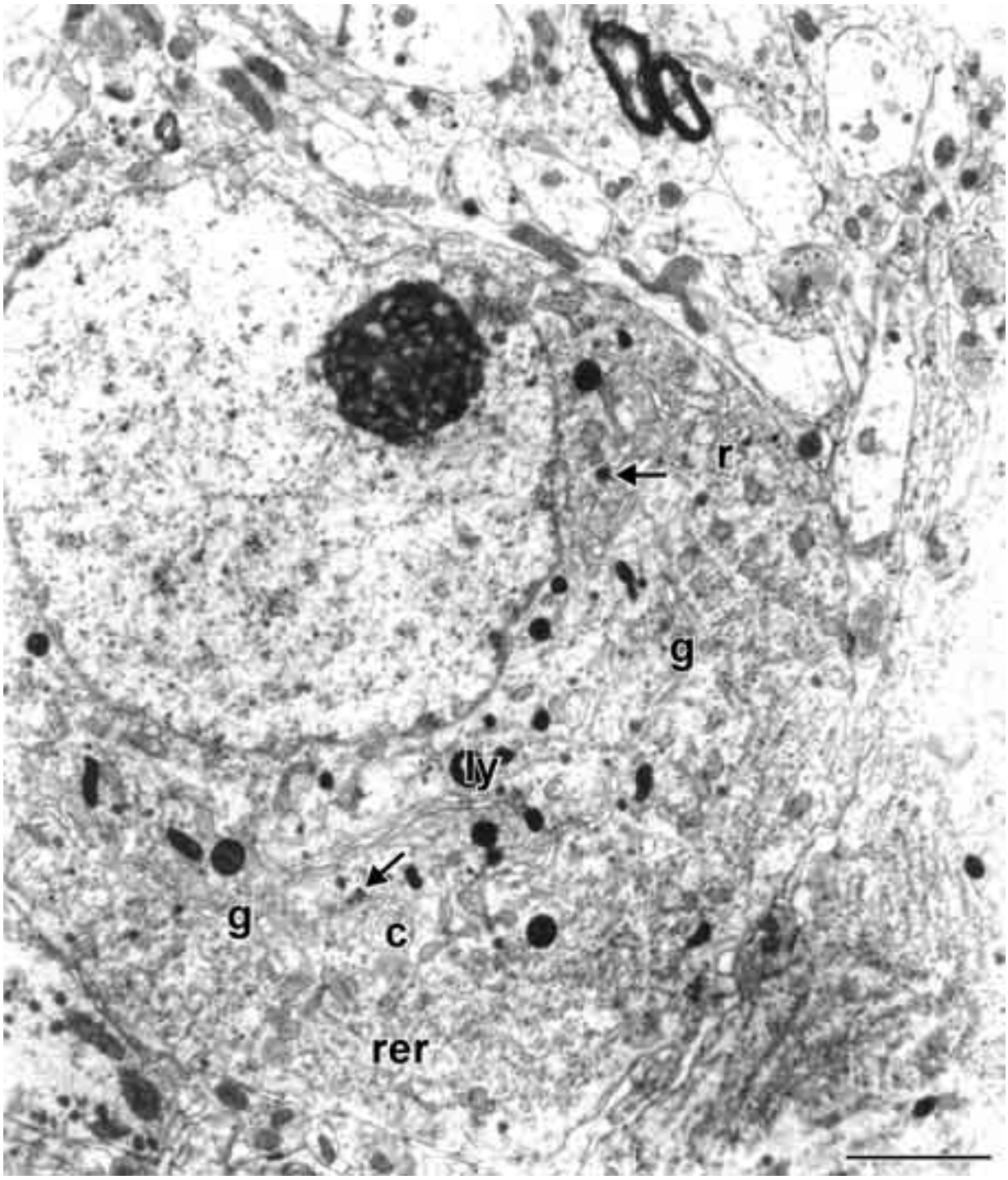


Figure 3. Young rat fasted for 48 h and refed for 24 h. Expanded numerous Golgi complexes (g), sometimes circular in structure (c), short RER cisternae (rer), and polyribosomes (r) dominate in the cytoplasm. Several neurosecretory granules (arrows) and lysosome-like bodies (ly) can be observed. Bar: 2 μm.

cristae could be observed. Prominent nucleoli were often situated in apposition to the nuclear envelope (Fig. 2).

Group III (fasted for 48 hours and refed for 24 hours). The striking ultrastructural feature was the presence of abundant Golgi complexes in the form of long and curved cisternae, some of them forming circular structures (Fig. 3). As was the case with fasted rats, numerous neurosecretory granules were localised close to extensive Golgi complexes. RER was found in the peripheral cytoplasm in the form of short fragments. Polyribosomes were loca-

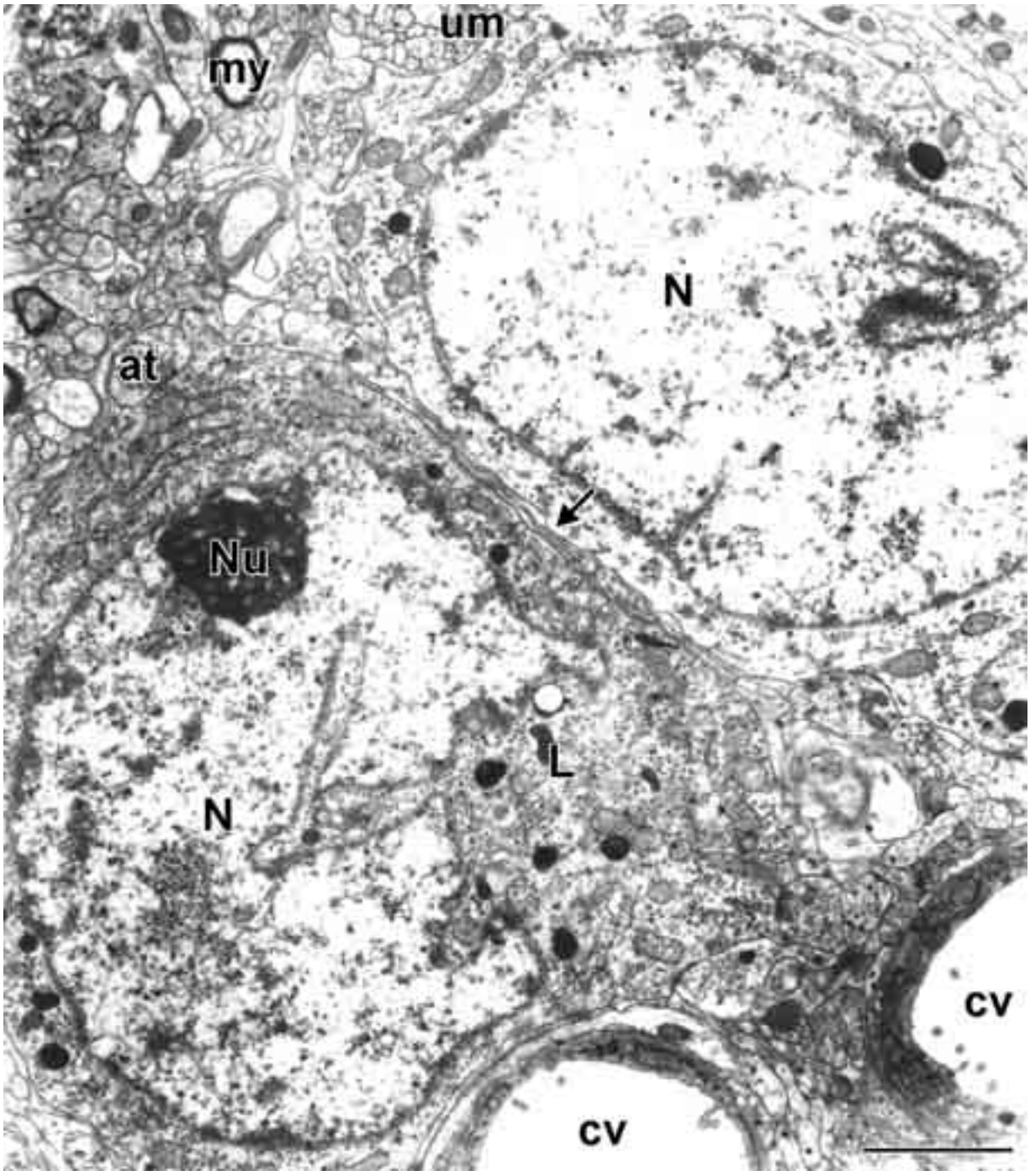


Figure 4. Control (normally fed) old rat. Two PVN neurons are separated by thin astroglial processes (arrow), large nuclei (N) show deep and branched invaginations of the nuclear envelope. A distinct nucleolus (Nu) is localised close to the nuclear envelope. Lipofuscin-like granules (L), capillary vessel (cv), axonal terminal (at), myelinated (my) and unmyelinated (um) axons. Bar: 2 μm.

lised mainly at the cell periphery (Fig. 3). The appearance of the nucleoli was less compact than in the fed or fasted rats (Fig. 3).

Old animals

Group I (fed control). In contrast to those of young rats, the magnocellular PVN neurons of the old animals showed deep and branched invaginations of the nuclear envelope that often contained organelles such as polyribosomes, RER, and mitochondria (Fig. 4). Besides lysosomes, lipofuscin-like granules (residual bodies) were present in the cytoplasm. Golgi complexes of typical appearance and neurosecretory granules were located in the perinu-

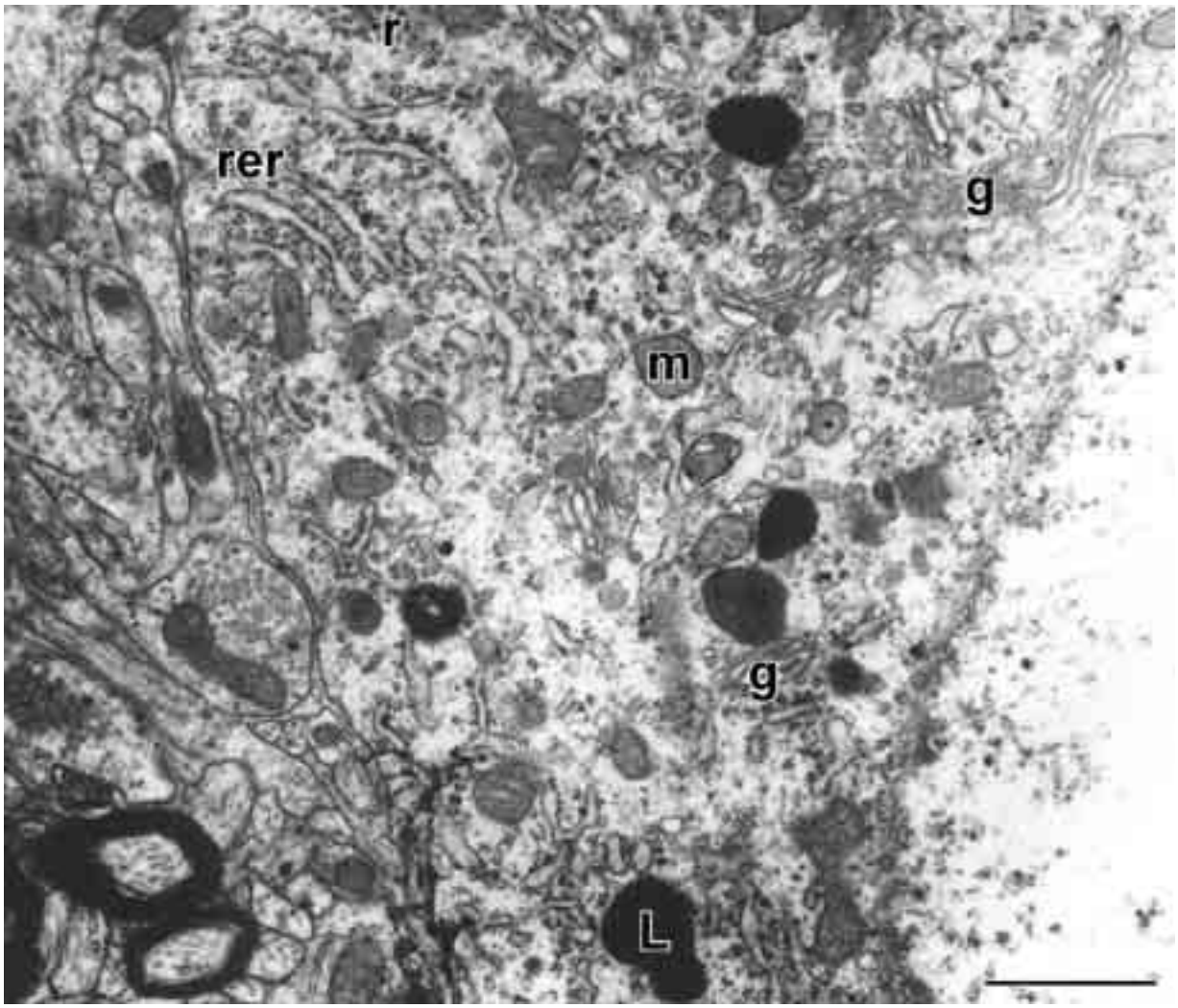


Figure 5. Control (normally fed) old rat. Part of the perikaryon contains several Golgi complexes (g), short fragments of RER cisternae (rer) and polyribosomes (r) localised mainly in the cell periphery. Mitochondria (m) and residual bodies (L) are scattered inside the cytoplasm. Bar: 1 μm.

clear cytoplasm. As was the case with young rats, fragmented RER cisternae were localised mainly at the cell periphery (Fig. 5).

Group II (fasted for 48 hours). As with young fasted rats food deprivation resulted in a vast expansion of Golgi complexes that were arranged parallel to the nuclear envelope. Prolonged Golgi cisternae were regularly accompanied by small vesicles and neurosecretory granules (Fig. 6, 7). Numerous polyribosomes were uniformly distributed in the cytoplasm. In contrast to the neurons of fed old rats, RER cisternae were localised between the nuclear envelope and Golgi complexes (Fig. 6).

Group III (fasted for 48 hours and refed for 24 hours). There were no significant differences in the appearance of PVN neurons of refed old rats as compared to fasted ones. However, many prolonged and sometimes branched RER cisternae were distributed in the peripheral cytoplasm (Fig. 8).

DISCUSSION

The hypothalamic paraventricular nucleus takes part in the regulation and coordination of many important physiological processes such as water homeostasis, autonomic functions, control of food intake and energy homeostasis. Rat PVN can be subdivided topographically into 2 divisions: magnocellular PVN that occupies one third of the total area of PVN, and parvocellular PVN (pPVN) that constitutes the remaining part of PVN [7]. Parvocellular neurosecretory cells of PVN release hypophyseotropic hormones (CRH, TRH, and somatostatin) at the median eminence, whereas magnocellular neurons release vasopressin and oxytocin in the posterior lobe of the

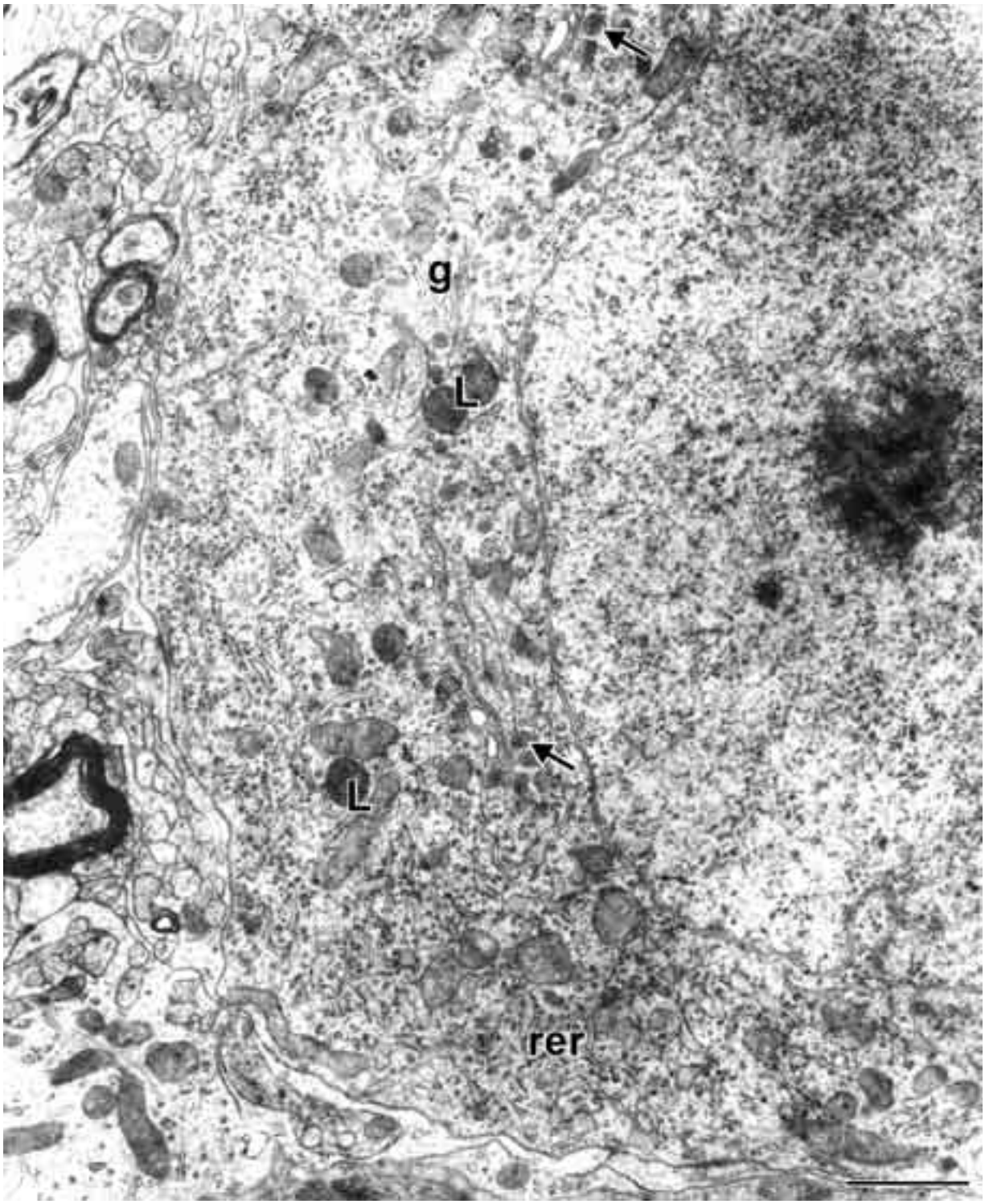


Figure 6. Old rat fasted for 48 h. RER cisternae (rer) in a form of short slightly dilated fragments are situated towards the periphery. In well developed Golgi zones (g) numerous small vesicles and dark neurosecretory granules (arrows) are visible. Residual bodies (L). Bar: 1 μm.

pituitary gland [2]. PVN neurons receive dense innervation from neurotransmitter and neuropeptide neurons, including those involved in the control of food intake and the regulation of energy homeostasis [6]. Receptors for neuropeptides (orexigenic neuropeptide Y, AgRP — Agouti-Related Protein, orexin, and anorexigenic POMC/aMSH, and CART — Cocaine and Amphetamine Related Transcript) and peripheral hormones (leptin, insulin) have been shown to exist in the PVN neurons [6, 12]. The neurons which

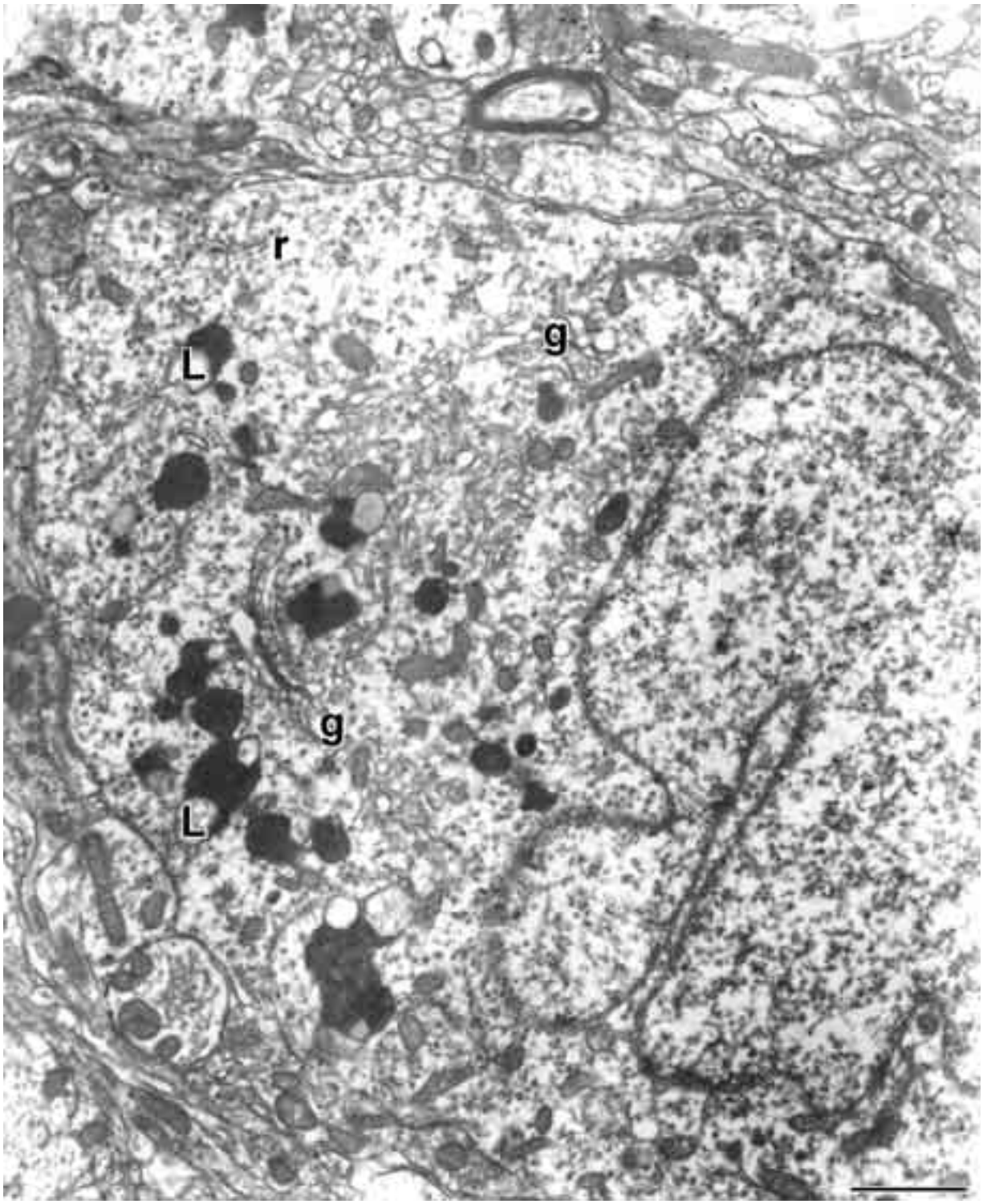


Figure 7. Old rat fasted for 48 h. Extensive Golgi complexes (g) with many vacuoles. Polyribosomes (r) localised mainly in the cell periphery. Multiform residual bodies (L). Bar: 1 μm.

release these peptides reside mostly in close proximity to PVN, for instance in the arcuate nucleus and lateral hypothalamus [17].

In an attempt to explain the lower food intake of refed old rat, we decided to investigate the ultrastructure of PVN neurons. Our electron microscopic studies were restricted to the magnocellular part of this nucleus, as it had been shown that oxytocin inhibited food intake in rats [1]. The ultrastructure of this division of PVN has not previously been charac-

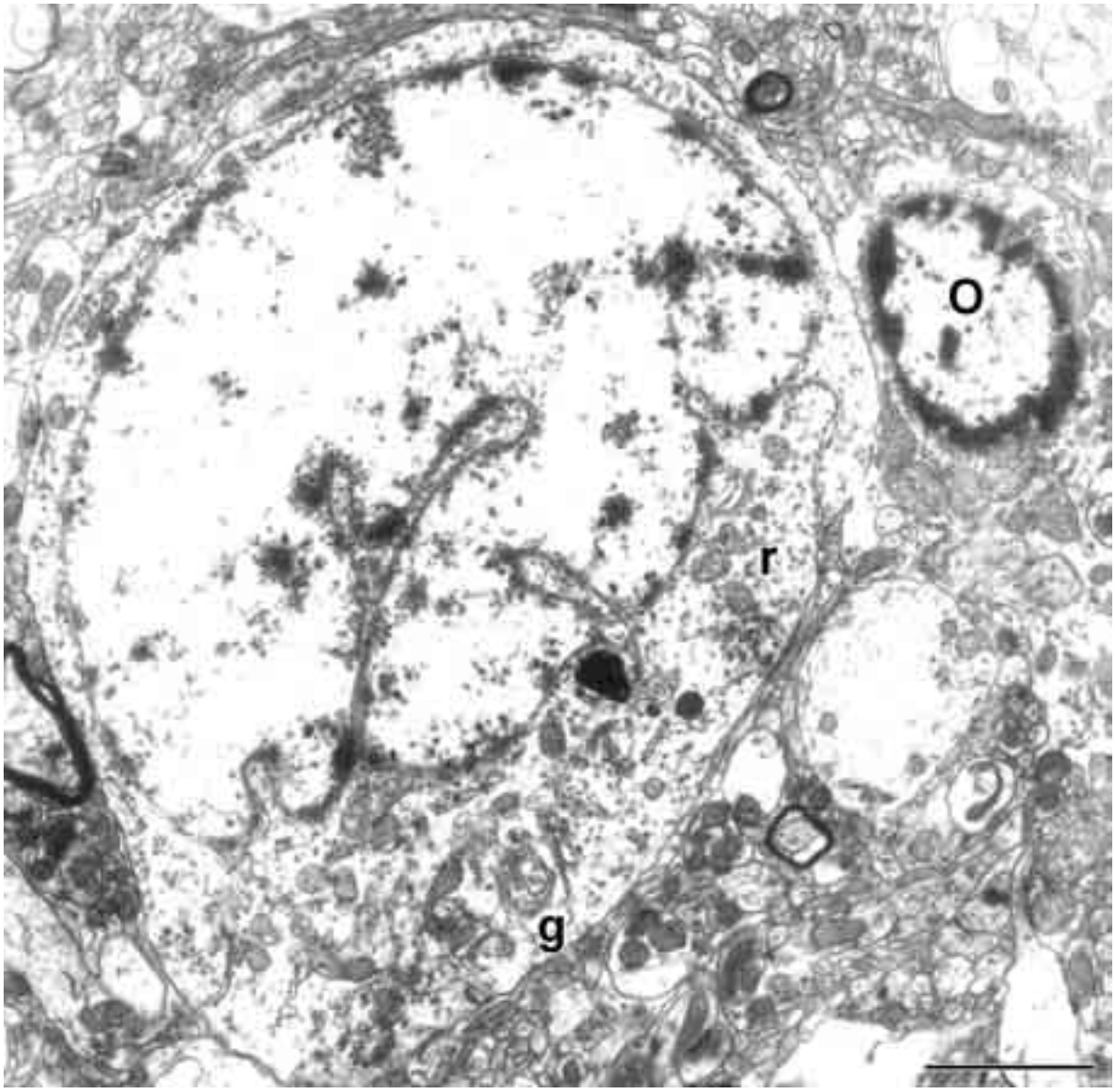


Figure 8. Old rat fasted for 48 h and refed for 24 h. Expanded Golgi complexes (g); polyribosomes (r) randomly dispersed in the cytoplasm. Oligodendrocyte (O). Bar: 2 μm.

terised in old rats, either in normal conditions or in the fasting state.

Our characterisation of the ultrastructure of PVN magnocellular neurons in normal young rats corresponds to the findings reported by other authors [5, 15, 19] who also noted the large nucleus with prominent and multiple nucleoli, extensive Golgi complexes and abundant RER and numerous polyribosomes. The mPVN neurons of old rats differed from those of young ones by the presence of multiple residual bodies (probably containing lipofuscin granules) and deep invaginations of the nuclear envelope that prominently increased the nucleo-cytoplasmic surface. These invaginations and the closer apposition of nucleoli to the nuclear envelope seem to be characteristic of the hypothalamic neurons of old rats, as we also observed them in VMN neurons [11].

The effect of fasting on the ultrastructure of PVN neurons has not yet been described. Our findings indicate that in both age groups the secretory activity of mPVN neurons increases after two days of food deprivation. Further immunohistochemical studies may explain the exact nature of the secretory product(s). We showed that fasting with an unrestricted water supply resulted in different ultrastructural changes in mPVN neurons from those induced

in the supraoptic hypothalamic neurons of young rats by dehydration and rehydration (an increase in the number of nucleoli and their margination within nuclei) [4].

Our results suggest that the activation of mPVN neurons in fasted young and old rats, reflected in the expansion of the Golgi apparatus and RER cisternae, persisted after 24 hours of refeeding. Thus the dramatic alteration of the nutritional state (the fasted-to-fed transition) was not reflected in change to the ultrastructure of the mPVN neurons. However, this observation does not preclude alteration in the functioning of mPVN neurons under the conditions of reinstated food supply. The use of *in situ* hybridisation and immunohistochemical and stereological analyses might explain whether the synthesis of neuropeptides characteristic for mPVN neurons such as oxytocin and vasopressin was activated by the alterations in the nutritional state. It may, however, be concluded that at the ultrastructural level the process of ageing does not change the responsiveness of mPVN neurons to fasting-refeeding.

REFERENCES

1. Arletti R, Benelli A, Bertolini A (1990) Oxytocin inhibits food and fluid intake in rats. Physiol Behav, 48: 825–830.
2. Cone RD, Low MJ, Elmquist JK, Cameron JL (2003) Neuroendocrinology. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS (ed.). William's textbook of endocrinology. 10th ed., Saunders, Philadelphia, pp. 83–176.
3. Grill HJ, Kaplan JM (2002) The neuroanatomical axis for the control of energy balance. Front Neuroendocrinol, 23: 2–40.
4. Hatton GI, Walters JK (1973) Induced multiple nucleoli, nucleolar margination, and cell size changes in supraoptic neurons during dehydration and rehydration in the rat. Brain Res, 59: 137–154.
5. Kalimo H (1971) Ultrastructural studies on the hypothalamic neurosecretory neurones of the rat. I. The paraventricular neurones of the non-treated rat. Z Zellforsch, 122: 283–300.
6. Kalra SP, Dube MG, Pu S, Xu B, Horvath TL, Kalra PS (1999) Interacting appetite-regulating pathways in the hypothalamic regulation of body weight. Endocr Rev, 20: 68–100.
7. Kiss JZ, Palkovits M, Zaborszky L, Tribollet E, Szabo D, Makara GB (1983) Quantitative histological studies on the hypothalamic paraventricular nucleus in rats. I. Number of cells and synaptic boutons. Brain Res, 262: 217–224.
8. Kmieć Z, Kotlarz G, Myśliwski A, Wyrzykowska M, Pokrywka L (2002) Thyroid C-cell function during fasting and refeeding of young and old rats. Folia Histochem Cytobiol, 40: 185–186.
9. Kmieć Z, Kotlarz G, Śmiechowska B, Myśliwski A (1998) The effect of starvation and refeeding on thyroid follicule structure and thyroid hormone levels in young and old rats. Arch Gerontol Geriatr, 26: 161–175.
10. Kmieć Z, Myśliwski A, Wyrzykowska M, Hoppe A (2001) The effect of fasting and refeeding on serum parathormone and calcitonin concentrations in young and old male rats. Horm Metab Res, 33: 276–280.
11. Kubasik-Juraniec J, Kmieć Z, Tukaj C, Adamowska A, Kotlarz G, Pokrywka L, Myśliwski A (2003) Ultrastructure of the ventromedial hypothalamic nucleus in fasted and refed young and old rats. Folia Morphol, 62: 89–98.
12. Leibowitz SF, Sladek C, Spencer L, Tempel D (1988) Neuropeptide Y, epinephrine, and norepinephrine in the paraventricular nucleus: Stimulation of feeding and release of corticosterone, vasopressin and glucose. Brain Res Bull, 21: 905–912.
13. Myśliwski A, Kmieć Z (1992) Effect of aging on glycogen synthesis in liver of starved refed rats. Arch Gerontol Geriatr, 14: 85–92.
14. Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic Press, New York.
15. Pilgrim C (1969) Morphologische und funktionelle Untersuchungen zur Neurosekretbildung. Ergebn Anat Entwickl-Gesch, 41: 1–79.
16. Reynolds ES (1963) The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J Cell Biol, 17: 208–213.
17. Schwartz MW, Woods S.C., Porte JrD, Seeley RJ, Baskin DG (2000) Central nervous system control of food intake. Nature, 404: 661–671.
18. Watson ML (1958) Staining of tissue sections for electron microscopy with heavy metals. J Biophys Biochem Cytol, 4: 475–478.
19. Zambrano D, de Robertis E (1968) The effect of castration upon the ultrastructure of the rat hypothalamus I. Supraoptic and paraventricular nuclei. Z Zellforsch, 86: 487–498.