Histol Histopathol (1999) 14: 1093-1099

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Size and degeneration increase in herring bodies during aging in hamsters

A. Navarro, J. Tolivia and M. Alvarez-Uría

Department of Morphology and Cell Biology, Faculty of Biology and Medicine, University of Oviedo, Oviedo, Spain

Summary. The hypothalamo-neurohypophysial tract of young, adult and aged male hamsters was studied at lateral and ventral regions of hypothalamus by means of electron microscopy. Neurosecretory swelling axons (Herring bodies) were usually found as classically described containing abundant neurosecretory granules, mitochondria, few microtubules and profiles of smooth endoplasmic reticulum in all groups of age. However, in aged hamsters, starting at 18-month-old subjects, we observed that the size of some neurosecretory axons was highly increased. Autophagic and degenerative features were seen in the larger ones. These data could suggest abnormal axonal storage or axonal transport blocked during aging. The implications in the role of hypothalamus-neurohypophysial system during aging are discussed.

Key words: Aging, Electron microscopy, Axonal transport, Hypothalamus

Introduction

The transport of neurosecretory material of the hypothalamo-neurohypophysial system (HNS) occurs into large axons of magnocellular peptidergic neurons between the perikaryon origin and its ending in the neural lobe (for review see Kupfermann, 1991). The axons form three compartments: undilated axons; preterminal dilated "nerve swellings" (Herring bodies) and terminal "nerve endings" in the median eminence and neurohypophysis, according to Morris' nomenclature (1976). These Herring bodies were classified into another three types (Dellmann and Rodriguez, 1970; Polenov and Garlov, 1971): Type I, characterized by numerous neurosecretory granulated vesicles (NGVs); Type II, with many autophagic vacuoles, dense bodies, NGVs and empty vesicles; and Type III, is distingued by an extensive network of smooth endoplasmic reticulum (SER), mitochondria and a few NGVs. These authors hypothesized that type I Herring bodies serve as temporary storage site for NGVs. If arrest is of longer periods, autophagic events are initiated, causing the characteristic appearance of Type II Herring Bodies. Type III are considered to represent the beginning of return to a normal stage.

The HNS has been implicated in the diminished capacity to maintain water balance and the ability to respond to a salt-loading stimulus during aging (Sladek et al., 1981; Phillips et al., 1984; Goudsmit et al., 1988). Several possible hypotheses could solve this question: 1) a decrease in the biosynthesis of neuropeptides; 2) reduction in the axonal transport of these peptides; 3) decrease in the ability of the neurohypophysis to release hormones; and 4) decrease in kidney function. On the one hand, the literature states that changes during aging in the HNS produce a decrease in neurosecretory activity (Dorsa and Bottemiller, 1982; Calzá et al., 1990). However, no significant cell loss was shown in these nuclei during aging that could explain this suggested decreased secretory activity of HNS (Hsü and Peng, 1978; Peng and Hsü, 1982; Sartin and Lamperti, 1985; Goudsmit et al., 1990). On the other hand, the neuropeptide production by these nuclei has been reported unaltered or increased in old subjects (Fliers and Swaab, 1983; Davies et al., 1985; Silverman and Sladek, 1991; Lucassen et al., 1993; Navarro et al., 1997). The cause for the activation of neurons is as yet unknown, but a possible explanation is the loss of vasopressin binding sites in the kidney with aging (Ravid et al., 1985; Davies, 1987). The apparently divergent data in the synthesis, the storage and the neurohormone release may be the result of a reduction in axonal transport with age. In this respect, a significant difference of transport of newly synthesized neurohormones in young and old mice exists (Fotheringhan et al., 1991).

We intended to work on this hypothesis by ultrastructural study and quantitative methods, the hypothalamo-neurohypophysial tract in hamsters during the lifetime. The observations of fine structural changes in these large axons between young and old hamsters are

Offprint requests to: Dr. Ana Navarro, Departamento de Morfología y Biología Celular, Facultad de Biología y Medicina, Universidad de Oviedo, Julián Clavería s/n, Oviedo 33006. Spain. Fax: 348-5103618. e-mail: anavarro@correo.uniovi.es

described and discussed in the present paper.

Materials and methods

Experimental animals

Male Syrian hamsters (*Mesocricetus auratus*) from our closed colony established in the Morphology and Cellular Biology Department, Oviedo University, in 1985, were employed in the present study. Animal groups of 3, 6, 12, 18, 24 and 30 months of age were studied. Five specimens were used for each age, comprising a total of 30 hamsters. Since the average life span for our golden hamster is 1.6 years, our three oldest groups are considered aged. They were housed in a temperature-controlled room (20±2 °C) with photoperiod of 14:10h light/dark cycles and with free access to laboratory chow and water. Any animals with any evidence of macroscopic pathology (detected by a detailed autopsy carried out during the perfusion process) were excluded from the study.

Tissue treatment

Before sacrifice, animals were anaesthetized with sodium pentobarbital (10.5 mg/100 g body weight) and transcardially perfused with 4% glutaraldehyde and 1mM $CaCl_2$ in 0.05M cacodylate buffer at pH 7.3. After perfusion brains were removed and fixed in the same fixative for 18 hours at 4 °C. The hypothalamic area was dissected out and the tissue blocks were postfixed in a solution of 1% osmium tetroxide and 2% potassium ferrocyanide in 0.05M cacodylate buffer (pH 7.3) for 1 hour, dehydrated in a graded series of acetone and embedded in Durkupan-ACM (Fluka). Semithin sections, about 0.5 μm thick, were obtained and stained with the first step of Tolivia et al. (1994) method.

Ultrathin sections (70-90 nm) were obtained on an LKB ultramicrotome and were placed on 300-mesh copper grids with apertures measuring 61x61. They were contrasted with uranyl acetate and lead citrate, and examined with a Zeiss EM 109.

For the quantitative study in electron microscopy, a total of three sections were taken for each subject (no less than 100 μm apart to prevent analysis of profiles of the same axon). Three randomly selected areas of parenchyma in each ultrathin section were selected and scanned at x3,000. Herring bodies were photographed in cross-sectional orientation and only profiles of axons with a complete outline were study. Data were expressed as mean ± S.D (Standard deviation).

Results

Prominent swelling (Herring bodies) and undilated neurosecretory axons could be observed in the lateral and ventral surface of the hypothalamus including median eminence in all age groups of hamsters. Examination of these areas confirmed differences in the size of the axons probably depending of synthesis or secretory cycles. Measurements of Herring bodies usually varied from 20 to 200 μm^2 in transectional area in young hamsters. Most of these large swellings were unmyelinizated axons, although occasionally they were surrounded by myelin layer. The normal Herring bodies, usually found contained abundant neurosecretory granules (NSGs), mitochondria, few microtubules and profiles of smooth endoplasmic reticulum in all age groups.

However, already in the 18-month-old hamsters, larger dilated neurosecretory axons appeared in the ventral surface of the hypothalamus, most of them just lateral to the rostral part of the median eminence. As seen with light microscopy, these dilated axons could be identified at the electron microscopic level as huge Herring bodies. The average cross-sectional area of these Herring bodies was 407±91.14 μm^2.

An irregular profile against the round or oval profile of normal Herring bodies was another common characteristic of these huge neurosecretory axons (Figs. 1, 2). The axonal plasmalemma presented deep invaginations, sometimes with small processes (Fig. 3A,B). In addition, the presence of normal Herring bodies surrounding or in direct apposition with the huge neurosecretory processes, normally appeared (Figs. 1, 2). The dilated processes contained a large accumulation of neurosecretory granules with different size and density. The number of autophagic vacuoles, dense bodies, dense lamellar bodies and swelling mitochondria was higher than normal (Fig. 3D). No abnormal proliferation of the smooth endoplasmic reticulum or filamentous accumulations was observed.

These huge Herring bodies that we have found in aged hamsters presented a higher or lower grade of degeneration. Different patterns of degeneration could be seen between peripheral and central areas (Fig. 2). Central areas presented more degenerated organelles than peripheral areas, where NSGs formed conserved aggregations (Fig. 3C,D).

No macrophage or microglial reaction could be seen in the nearby neuropil. A normal relationship between axolemma and the apposing astroglial membrane was seen (Fig. 2).

The axonal transectional area presented an age variation. The Herring bodies of small size (<200 μm^2) are the most abundant and constituted 89% of the total axonal profile measured under the age of 12 months. Medium sized axons (200-400 μm2) represented more than 40% in the12-18-month-old groups. The giant axons (>400 μm2) only appeared in the 18-month-old groups and could represent 20% of axons measured (Fig. 4).

Discussion

The above described ultrastructural and quantitative characteristics found in Herring bodies of ventral and lateral surfaces of the hypothalamus in older hamsters

support the idea that frequently some neurosecretory axons suffer increased axonal size and degeneration during aging. Watkins and Choy (1980) have reported changes in the appearance of the HNS during aging in rats. The neurosecretory axons were larger, more varicose, and the beaded-fibre structures were less delicately defined during aging. Similar changes have been reported in dogs by Scharrer (1954).

Nerves swelling along the axons have been considered the result of local variation in the transport

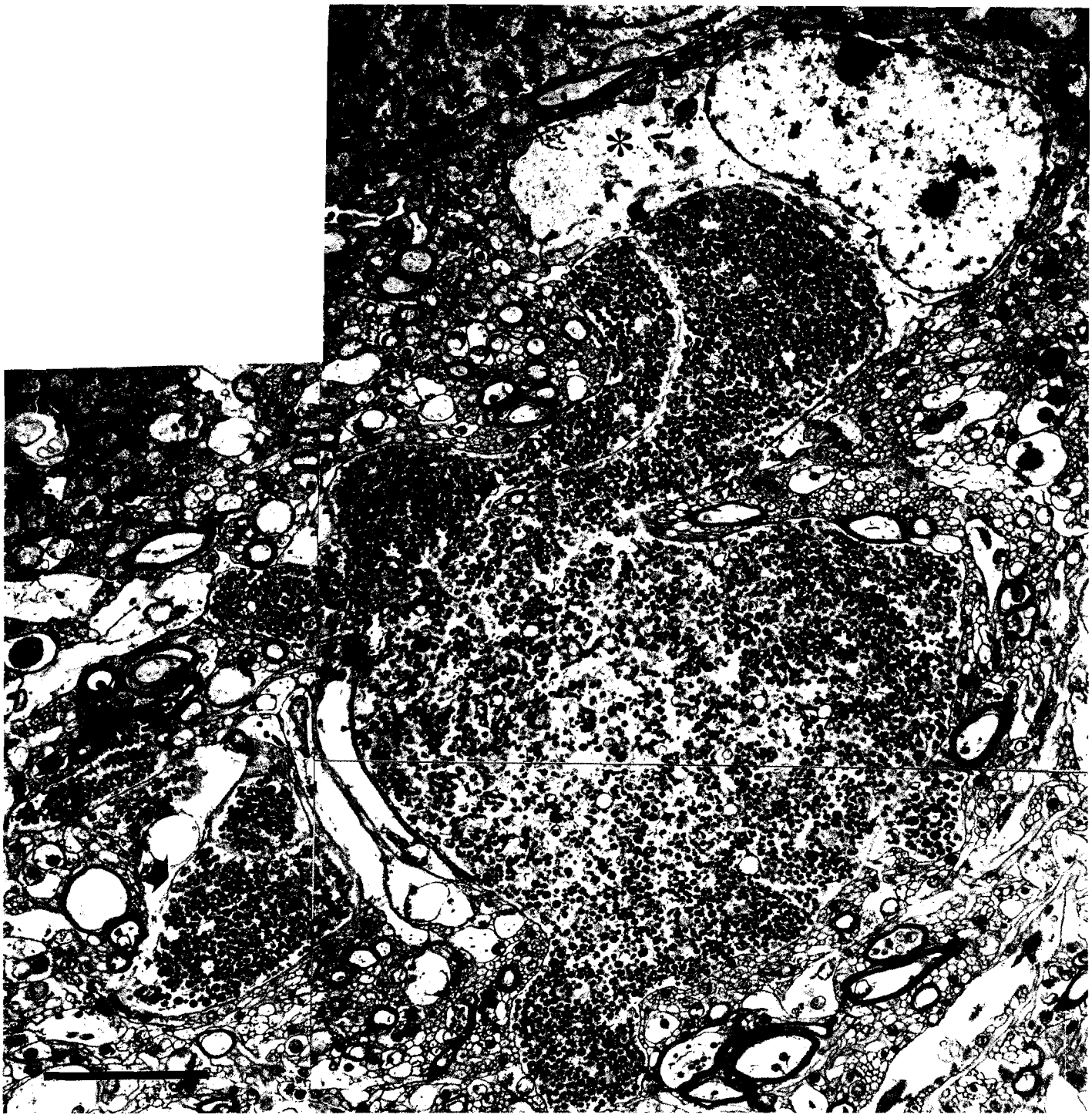

Fig. 1. Lateral hypothalamus at low magnification with a large Herring body of a 18-month-old hamster closely to an astrocyte (asterisk). Bar: 5 μm.

velocity, the undilated axon segments being regions of fast transport while swelling is conceived as an area of slow transport, temporary storage or disposal of vesicles (Morris et al., 1978; Peña et al., 1988). For these reasons, one possible explanation to the huge Herring bodies that we found in old hamsters could be an increased neurohormone synthesis in the HNS with a reduction in neuroaxonal transport with age.

Some works have proved that there is an increase (Davies et al., 1984; Silverman and Sladek, 1991) or a maintenance (Navarro et al., 1997) of peptidergic synthesis in magnocellular neurons of hypothalamus during aging. However, stereomorphometric analyses show a conspicuous depletion of NSGs from the Herring bodies and axon terminals in the neurohypophysis of aged male rats (Rechardt and Hervonen, 1982). A decrease in the ability of the neurohypophysis to release the AVP has also been shown (Davies, 1987). Davies et al. (1990) have identified reductions in neurohypophysis in the volume of the axonal endings and swelling of normally hydrated old mice and a delay in restocking NSGs when osmotically stressed aged animals were rehydrated.

These contradictory data could be explained by a reduction in neuronal transport with age that could produce an extraneurohypophysial storage within the

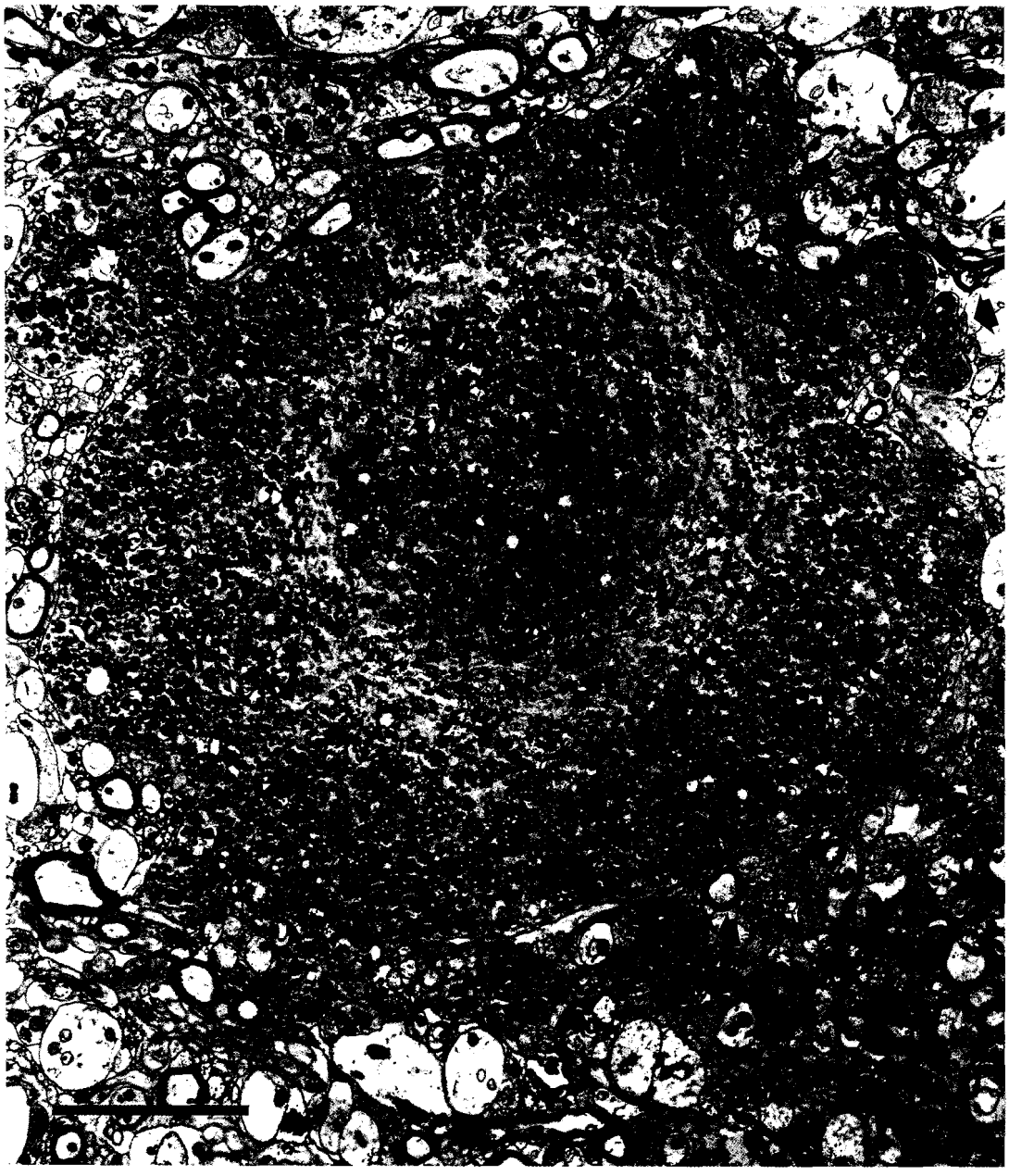

Fig. 2. Low-power electron micrograph of one large Herring body of a 24- month-old hamster with small undilated neurosecretory axons (arrow). Bar: 5 μm.

Herring bodies running in the lateral and ventral hypothalamus. Significant reductions in the rate of neuroaxonal transport of AVP from SON to the neurohypophysis has been reported by Fotheringham et al. (1991). Despite the specific mechanism vesicle transport has been shown to be significantly slower in older animals and that some axons may be more affected than others (Viancour and Kreiter, 1993). Presumably,

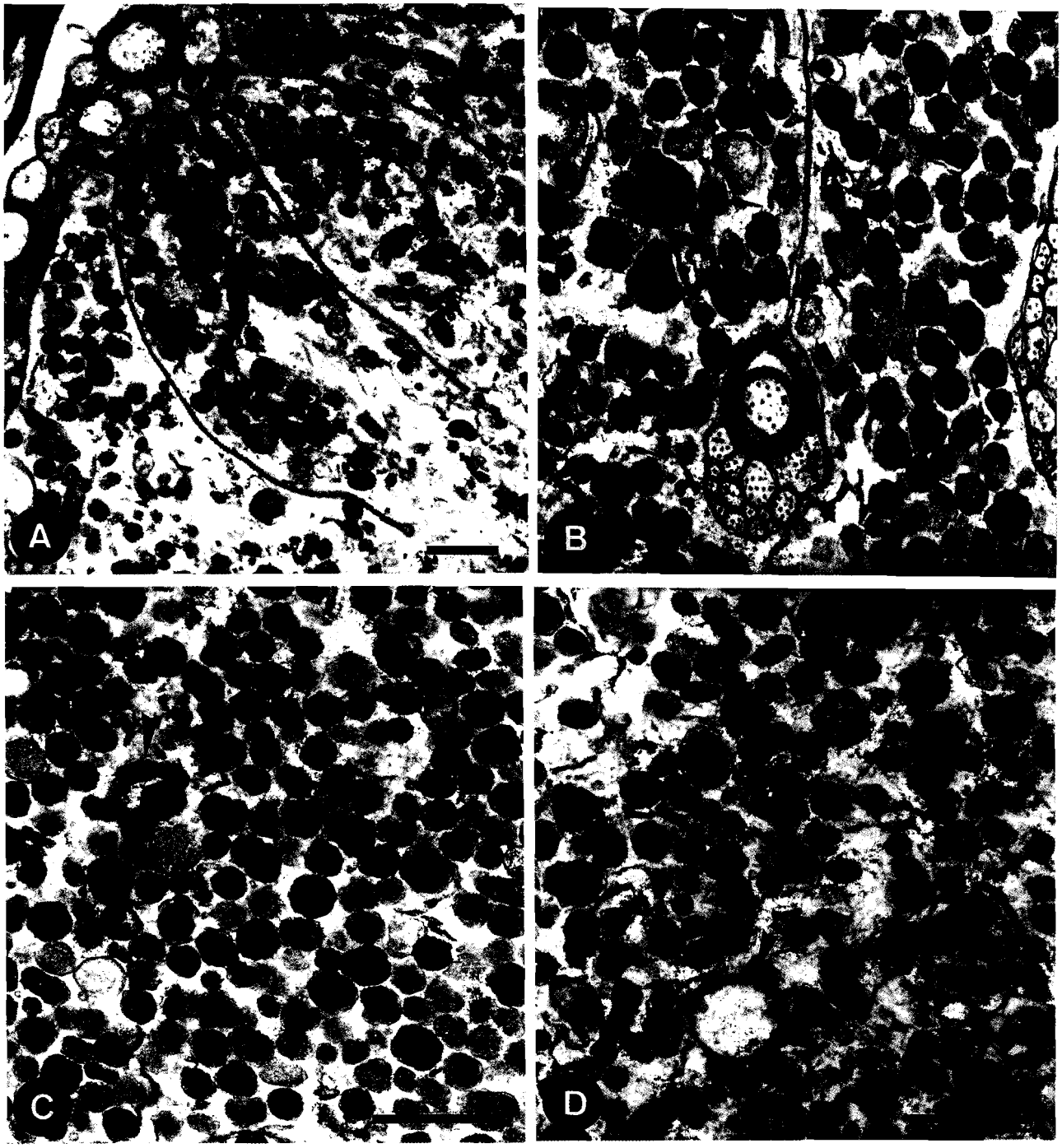

Fig. 3. Some details of dilated Herring bodies are shown in micrographs. **A.** Plasmalemma invaginations from a 24-month-old hamster. Bar: 1 μm. **B.** Myelinated and non-myelinated neurites within one plasmalemma invagination. Bar: 0.5 μm. **C.** Conserved mitochondria (arrowhead) and neurosecretory granules in a peripheral portion. Bar: 0.5μm. **D.** A degenerated central portion with amounts of multivesiculated bodies, few neurosecretory granules and swelling mitochondria (arrowhead). Bar: 0.5 μm.

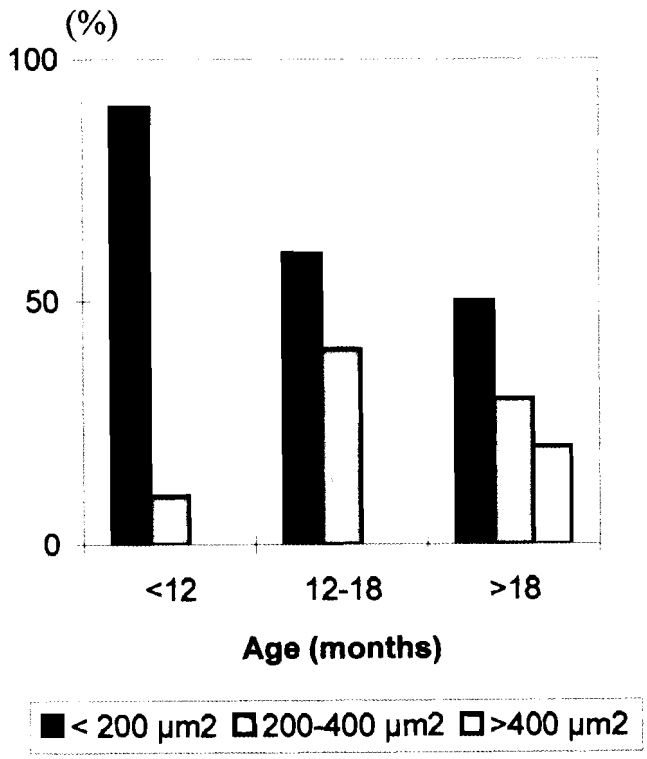

Fig. 4. Graph showing axonal cross-sectional area distribution during aging.

large vesicles are most affected by age. On the other hand, the number and rate of neurofilaments exported from the neuron body are decreased during aging and could produce increases in the diameter of the axon (Saitua and Alvarez, 1988; Inestrosa and Alvarez, 1988). This fact could explain the huge size of the Herring bodies found in old hamsters. Thus, it has been reported that swelling processes contain fewer microtubules or microfilaments than undilated ones (Morris and Pow, 1991). Hoffman et al. (1984) have also reported that a change in the rate of cytoskeletal transport can affect the diameter of the axon. Microiontophoretic ejection of vinblastine or colchicine (which block axonal transport) into the hypothalamus-neurohypophyseal tract produces large axon swellings that at advanced post ejection times, appear degenerated (Chang and Dellmann, 1984). Thus, these very large Herring bodies could represent areas of slow transport where NSGs are temporally stored and could be removed by transport or degradation processes. In the same way, other authors have suggested that not all the swellings along the axons may have the same biochemical and functional features (Trembleu et al., 1994).

Based on the morphological characteristics of the Herring body types reported by Dellmann and Rodriguez (1970) and some induced experimental blocks of axonal transport in neurosecretory axons in frogs (Chang and Dellmann, 1984), we suggest that aging also causes degeneration of neurosecretory axons, probably due to a long arrest of axonal transport. The inability of the axoplasmic constituents to move distantly is responsible for the accumulation and the autophagic vacuoles and secondary lysosomes are indicatives of catabolic activity in the large and giant Herring bodies found in the oldest hamsters. The fact that no accumulations of SER networks and associated vesicles were found in any age group, could indicate that the arrest is not temporally and no regeneration process occurs. Due to the fact that in previous works degenerated neurons have been not found in magnocellular nuclei (Navarro et al., 1997), we suggest that these large swellings of neurosecretion could have lost continuity with the axon and often undergo degeneration. This phenomena can eventually occur in dystrophic neurites when a disturbance in the metabolism of the axoplasm exists.

In spite of these changes observed in the present study, the ability of the HNS to release hormones in response to a stimulus may be not impaired, due to the large amount of hormones stored in non-affected terminal axons of neurohypophysis.

Acknowledgements. This work was supported by FIS (96/1546; 99/1316) and the Universidad de Oviedo (DF95/218-5) grants. We would like to thank Carlos Villa and Fernando Jañez for technical assistance in electron microscopy.

References

Calzá L., Giardino L., Velardo A., Battistini N. and Marrama P. (1990). Influence of aging on the neurochemical organization of the rat paraventricular nucleus. J. Chem. Neuroanat. 3, 215-231.

Chang A.C. and Dellmann H.D. (1984). A fine structural study of the formation of temporary swellings (Herring bodies) and reversible degeneration of neurosecretory axons following microiontophoretic ejection of vinblastin into the hypothalamo-neurohypophysial tract of the frog (*Rana pipiens*). Exp. Brain Res. 53, 357-369.

Davies I. (1987). Ageing in the hypothalamo-neurohypophysial-renal system. Comp. Gerontol. 1, 12-23.

Davies I., Fotheringham A.P. and Roberts C. (1984). The effect of osmotic challenge and subsequent rehydration on the aging hypothalamo-neurohypophyseal system: A quantitative morphological study of the supraoptic nucleus. Mech. Ageing Dev. 26, 299-310.

Davies I., Goddard C., Fotheringham A.P., Moser B. and Faragher E.B. (1985). The effect of age on the control of water conservation in the laboratory mouse: Metabolic studies. Exp. Gerontol. 20, 53-66.

Davies I., Davidson C., Goddard C., Moser B., Faragher E.B., Morris J.A. and Wilkinson A. (1990). The ageing hypothalamo-neurohypophyseal system. An analysis of the neurohypophysis in normal hydration, osmotic loading and rehydration. Mech. Ageing Dev. 51, 157-178.

Dellmann H.D. and Rodriguez E.M. (1970). Herring bodies: an electron microscopic study of local degeneration and regeneration of neurosecretory axons. Z. Zellforsch. 111, 293-315.

Dorsa D.M. and Bottemiller I. (1982). Age-related changes of vasopressin content of microdissected areas of the rat brain. Brain Res. 242, 151-156.

Fliers E. and Swaab D.F. (1983). Activation of vasopressinergic and oxytocinergic neurons during aging in the Wistar rat. Peptides 4, 165-170.

Fotheringhan A.P., Davidson Y.S., Davis I. and Morris J.A. (1991). Age-associated changes in neuroaxonal transport in the hypothalamo-neurohypophysial system of the mouse. Mech. Ageing Dev. 60, 113-121.

Goudsmit E., Fliers E. and Swaab D.F. (1988). Vasopressin and oxytocin excretion in the Brown Norway rat in relation to aging, water metabolism and testosterone. Mech. Ageing Dev. 44, 241-252.

Goudsmit E., Hofman M.A., Fliers E. and Swaab D.F. (1990). The supraoptic and paraventricular nuclei of the human hypothalamus in relation to sex, age and Alzheimer's disease. Neurobiol. Aging 11, 529-536.

Hofman P.J., Griffin J.W. and Price D.L. (1984). Control of axonal caliber by neurofilament transport. J. Cell Biol. 99, 705-714.

Hsü H.K. and Peng M.T. (1978). Hypothalamic neuron number of old female rats. Gerontology 24, 434-440.

Inestrosa N.C. and Alvarez J. (1988). Axons grow in the aging rat but fast transport and acetylcholinesterase content remain unchanged. Brain Res. 441, 331-338.

Kupfermann I. (1991). Hypothalamus and limbic system: Peptidergic neurons, homeostasis, and emotional behavior. In: Principles of neural science. Kandel E.R., Schwartz J.H. and Jessell T.H. (eds). Elsevier. New York. pp 735-749.

Lucassen P.J., Ravid R., Gonatas N.K. and Swaab D.F. (1993). Activation of the human supraoptic and paraventricular nucleus neurons with aging and in Alzheimer's disease as judged from increasing size of the Golgi apparatus. Brain Res. 632, 105-113.

Morris J.F. (1976). Hormone storage in individual neurosecretory granules of the pituitary gland: A quantitative ultrastructural approach to hormone storage in the neural lobe. J. Neuroendocrinol. 68, 209-224.

Morris J.F. and Pow D.V. (1991). Widespread release of peptides in the central nervous system: Quantification of tannic acid-captured exocytoses. Anat. Rec. 231, 437-445.

Morris J.F., Nordman J.J. and Dyball R.E.J. (1978). Structure-function correlation in mammalian neurosecretion. Int. Rev. Exp. Pathol. 18, 1-95.

Navarro A, Tolivia J. and Alvarez-Uría M. (1997). The magnocellular neurosecretory system of the hamster hypothalamus: An ultrastructural and morphologic study during lifetime. Mech. Ageing Dev. 97, 143-161.

Peng M.T. and Hsü H.K. (1982). No neuron loss from hypothalamic nuclei of old male rats. Gerontology 28, 19-22.

Peña P., Rodriguez E.M., Dellman H-D. and Schoebitz K. (1988). Effects of colchicine on the hypothalamo-neurohypophysial system of chronically salt-loaded rats. Neuroendocrinol. 47, 217-224.

Phillips P.A., Rolls B.J., Ledingham J.G.G., Morton J.J., Crowe M.J. and Wollner L. (1984). Reduced thirst after water deprivation in the elderly. New Engl. J. Med. 311, 753-759.

Polenov A.L. and Garlov P.E. (1971). The hypothalamo-hypophysial system in Acipenseridae. I. Ultrastructural organization of large neurosecretory terminals (Herring bodies) and axoventricular contacts. Z. Zellforsch 116, 349-374.

Ravid R., Swaab D.F. and Pool C.W. (1985). Immunocytochemical localization of vasopressin-binding sites in the rat kidney. J. Endocrinol. 105, 133-149.

Rechardt L. and Hervonen H. (1982). Ultrastructural changes in the neurohypophysis of the aged male rat. Cell Tissue Res. 226, 51-62.

Saitua F. and Alvarez J. (1988). Do axons grow during adulthood: A study of caliber and microtubules of sural nerve axons in young, mature and aging rats. J. Comp. Neurol. 269, 203-209.

Sartin J.L. and Lamperti A.A. (1985). Neuron numbers in hypothalamic nuclei of young, middle-aged and aged male rats. Experientia 41, 109-111.

Scharrer E. (1954). The maturation of the hypothalamo-hypophyseal neurosecretory system in the dog. Anat. Rec. 118, 437.

Silverman W.F. and Sladek Jr. J.R. (1991). Ultrastructural changes in magnocellular neurons from the supraoptic nucleus of aged rats. Dev. Brain Res. 58, 25-34.

Sladek C.D., McNeil T.H., Gregg C.M., Blair M.L. and Baggs R.B. (1981). Vasopressin and renin response to dehydration in aged rats. Neurobiol. Aging 2, 293-302.

Tolivia J., Navarro A. and Tolivia D. (1994). Polychromatic stain of epoxy semithin sections: a new and simple method. Histochemistry 10, 51-55.

Trembleu A., Morales M. and Bloom F.E. (1994). Aggregation of vasopressin mRNA in a subset of axonal swellings of the median eminence and posterior pituitary: Light and electron microscopic evidence. J. Neurosci. 14, 39-53.

Viancour T.A. and Kreiter N.A. (1993). Vesicular fast axonal transport rates in young and old rat axons. Brain Res. 628, 209-217.

Watkins W.B. and Choy V.J. (1980). The impact of aging on neuronal morphology in the rat hypothalamo-neurohypophysial system: An immunohistochemical study. Peptides 1, 239-245.

Accepted May 5, 1999