Histol Histopath (1994) 9: 259-262

Histology and Histopathology

Zinc in hypothalamus and hypophysis of the rat

C. Pérez-Castejón[1], A. Vera-Gil[1], M.J. Barral[1], M.J. Pérez-Castejón[2] and M. Lahoz[1]
[1]Department of Morphological Sciences, Faculty of Medicine, University of Zaragoza, Zaragoza and
[2]Department of Nuclear Medicine, Faculty of Medicine, Complutense University, Madrid, Spain

Summary. Zinc has been located using both histochemical and autoradiographic procedures in the neurons of the nuclei of the hypothalamic medial area and in some adenohypophisary cells. Some suggestions about the functional significance of the presence of Zn in these places are made.

Key words: Hypothalamus-Hypophysis, Zinc, ^{65}Zn, Histochemistry, Autoradiography

Introduction

The presence of Zn in the central nervous system (CNS) is a fact since it was demonstrated by using intravital dytizon (Maske, 1952; Fleischhauer and Horstman, 1957) and it was confirmed using silver sulphide staining (Timm, 1958a,b). Further evidence was achieved using ^{65}Zn autoradiography (Euler, 1962; Fujii, 1967; Hassler and Soremark, 1968) in the decade of the sixties. Also we have some experience in this subject using both histochemical and autoradiographical techniques together (Vera-Gil, 1974). The presence of Zn in the CNS was first described in rats and was later confirmed for humans (Frederickson et al., 1983).

In the past decade, Zn has also been related to the production, storage and release of prolactine (Judd et al., 1984; Jacobs and Lorenson, 1986; Martinez-Escalera et al., 1986). This fact was confirmed using histochemical and PIXE measures and related to the secretory granules and Golgi apparatus of the STH, ACTH and TSH cells (Thorlacius-Ussing, 1987). Further evidence about the exchange of metallic ions for ^{65}Zn in order to activate a metallopeptidase related to TRH has been adduced by Czekay and Bauer (1993).

Because of this, in the present paper we have realized an autoradiographical and histochemical study to look for the hypothetical link of Zn in the hypothalamus/hypophysis axis.

Offprint requests to: Prof. Dr. M. Carmen Pérez Castejón, Departamento de Ciencias Morfológicas, Facultad de Medicina, Universidad de Zaragoza, C/ Domingo Miral s/n, 50009 Zaragoza, Spain

Materials and methods

24 young adult Wistar rats weighing an average of 225 gr were used. They were divided into 2 groups.

The first one, consisting of 12 animals, was used to carry out the histochemical technique for location of heavy metals described by Timm (1958a,b). Timm's technique modified by Phil (1968), was also carried out for histochemistry at the level of semithin sections.

The second group, also consisting of 12 animals, was injected intraperitoneally with 0.5 ml of a saline solution of $^{65}ZnCl_2$ named ZAS and supplied by Nuclear Iberica, with a calibrated activity of 355 µcCi. The animals were killed on the 6th day postinjection. Samples of hypothalamus and hypophysis were used for autoradiography at conventional histology level. Fixation was in a Carnoy solution and the autoradio-graphic method was by dipping in Ilford K5 emulsion, 50% dissolved in double distilled water. Exposure was in black plastic boxes with a drying system, inside a refrigerator at 4 °C for 21 days. Kodak D19 was used as developer fixed and Hypamas. Fading and blackening controls were made in order to discard both positive and negative chemography.

Results

Both techniques, histochemistry and autoradiography, showed the presence of Zn in the ventromedial hypothalamic nucleus. The location was mostly in the neuronal bodies, as is seen with autoradiography (Fig. 1A). The labelling was continuous throughout the axons which form the hypophisary stalk, as far as the posterior lobe (Fig. 1B).

Histochemistry revealed positivity in hypophysis, as is shown in Fig. 2A,B. The reaction show the axons ending in a network-like pattern, near to the surface of the pars intermediae.

With semithin sections and using Phil's variation of the Timm's histochemical technique, histochemistry showed positivity in the cytoplasm of the secretory cells of the adenohypophysis similar to the description made by Thorlacius-Ussing (1987) (Fig. 2C).

Discussion

Being critical with our results, we may discuss in the first place the specificity of Timm's histochemical method. With regard to this, we think that after the careful reading of the papers by Danscher (1981, 1982, 1984a,b), Frederickson et al. (1987) and Frederickson (1989), the conclusion is that such inspecificity is only formal and that the silver sulphide technique really reveals Zinc.

On the other hand, we believe that the discussion about the bioavailability of Zn in neurons and the behaviour of ^{65}Zn in the CNS that was analysed by Frederickson (1989), must be focused on Kasarskis's criterium (1984). Our repeated experiences with ^{65}Zn autoradiography point this out, but only if the sacrifice

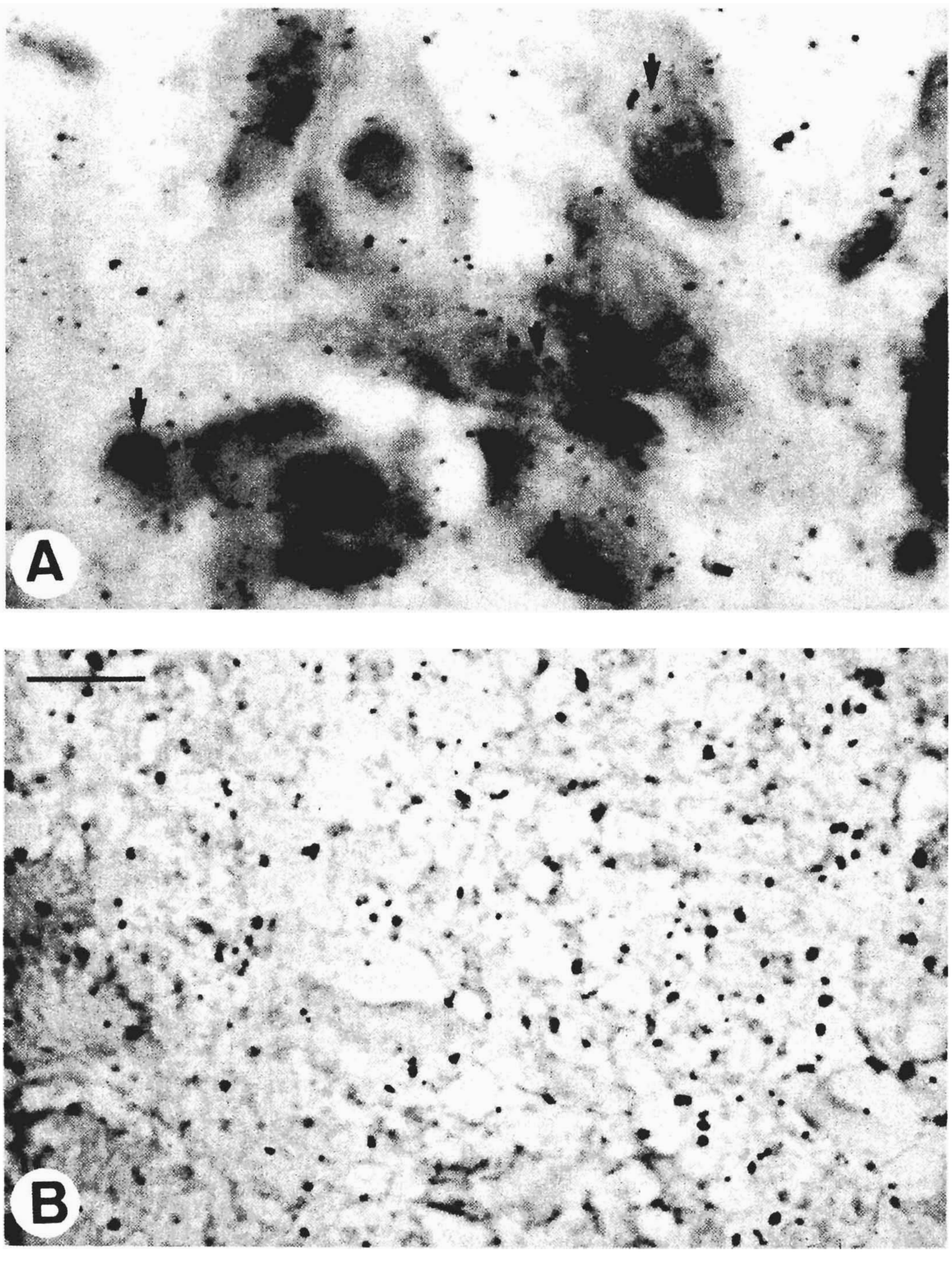
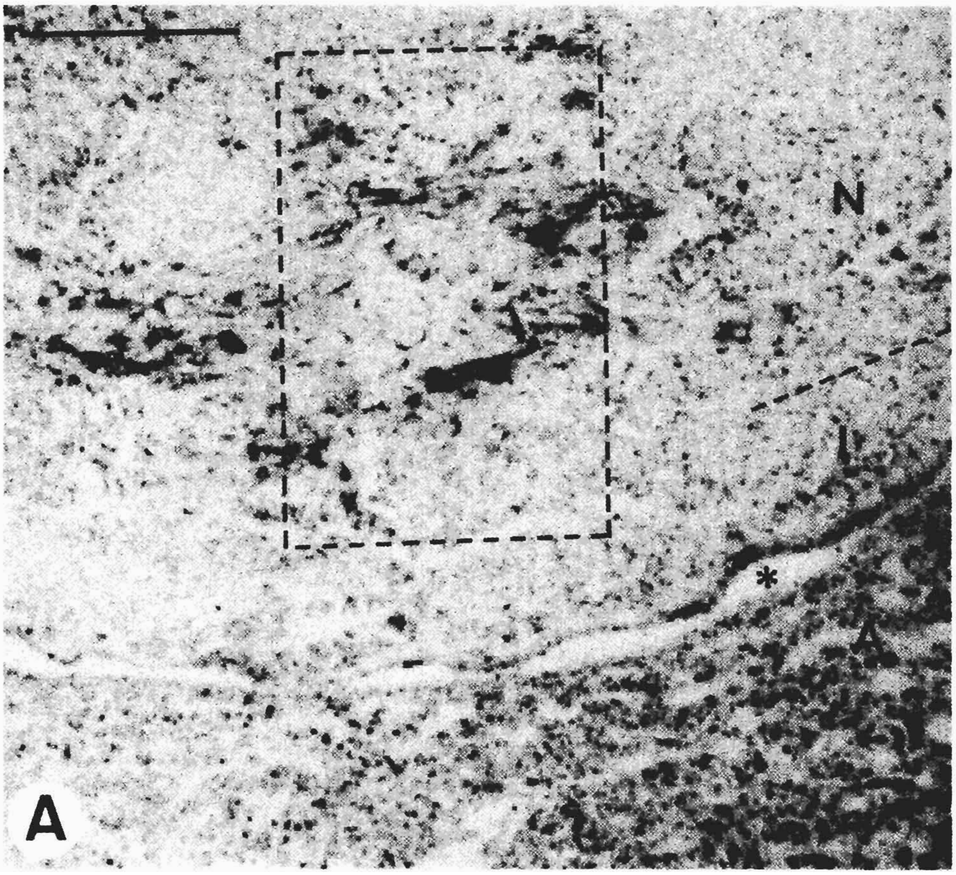


Fig. 1. ^{65}Zn autoradiography showing the uptake in the hypothalamus. **A.** ^{65}Zn positivity in the ventromedial hypothalamic nucleus, the arrows show some of the locations of the labelling. **B.** ^{65}Zn labelling in the infundibular stalk. Scale bar= 10 µm.



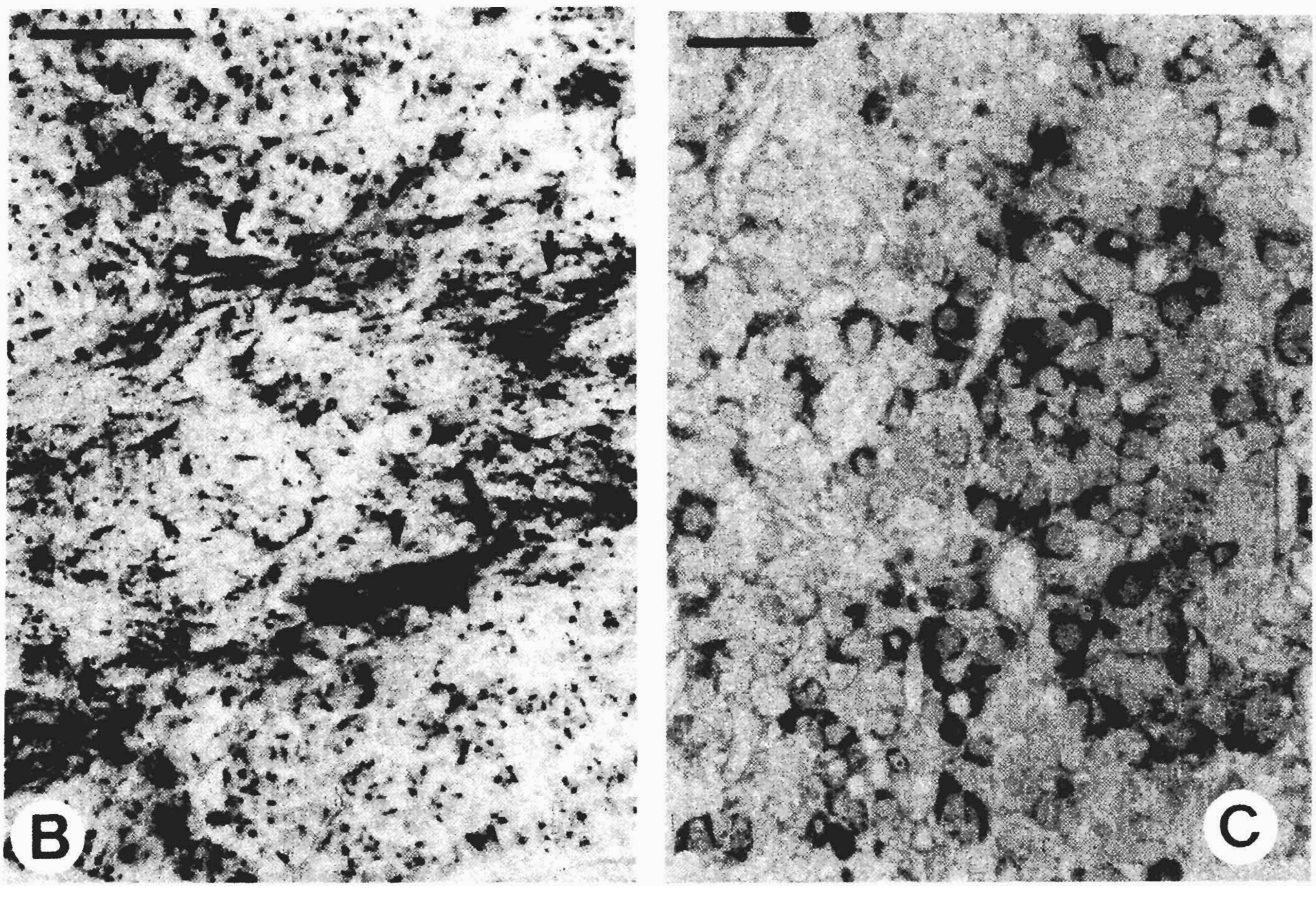


Fig. 2. Photomicrographs showing the Timm reaction in hypophysis. No other staining has been added for background. **A.** General view at low magnification. N: neurohypophysis; I: pars intermediae; *: grothe of the hypophysis; A: anterior lobe of the hypophysis. Scale bar= 100 µm. **B.** Higher magnification related to Picture A, showing the ending detail of positivity in the neurohypophysis. Scale bar= 40 µm. **C.** Semithin section (Phil's technique), showing the detail of the positivity in the adenohypophysis. Scale Bar= 20 µm.

of animals happens up to five days postinjection. Solving by these means the two main problems of the methods used, we suggest the following explanations for our findings.

In the CNS the presence of Zn has been related to very different roles such as: DNA and RNA synthesis, an active factor in the metabolism, storage and release of neurotransmitters, an ionic factor involved in the modulation of the neurotransmission related to Ca^{++} ion and NMDA receptors, and so on. We think that all of them are possible, but in our study we suggest that Zn is involved in the synthesis transport and probably in the release of the releasing hormones, acting in the same functions as are mentioned in the references.

Our adenohypophisary results confirm the above-mentioned references. After that, looking at the nature of this location, we believe that, from a functional point of view, these findings would suggest the relation of the role of Zinc in a similar form to its behaviour in other endocrine territories such as, for instance, the pancreas (McIssak, 1955).

References

Czekay G. and Bauer K. (1993). Identification of the Thyrotropin-Releasing-Hormone-degrading ectoenzyme as a metallopeptidase. Biochem. J. 290, 921-926.

Danscher G. (1981). Histochemical demonstration of heavy metals. A revised version of the sulphide silver method suitable for both light and electron microscopy. Histochem. 71, 1-16.

Danscher G. (1982). Exogenous selenium in the brain. A histochemical technique for light and electron microscopical localization of catalytic selenium bonds. Histochemistry 76, 281-293.

Danscher G. (1984a). Similarities and differences in the location of metals in rat brains after treatment with sodium sulphide and sodium selenide. In: The neurobilology of Zinc. (part A). Frederickson C.J., Howell G.A. and Kasarskis E.J. (eds). Alan R. Liss Inc. New York. pp 229-242.

Danscher G. (1984b). Do the Timm sulphide silver method and the selenium method demonstrate zinc in the brain? In: The neurobiology of Zinc. (part A). Frederickson C.J., Howell G.A. and Kasarskis E.J. (eds). Alan R. Liss Inc. New York. pp 273-287.

Euler (Von) C. (1962). On the significance of the high Zinc content in the hippocampal formation. In: Physiologie de l'hippocampe. Passouant P. (ed). Centre National de la Recherche Scientifique. Paris. pp 135-145.

Fleischhauer K. and Horstman E. (1957). Intravitale dithizonfarbung homologer felder der Ammonsformation von saugern. Z. Zellforsch. 46, 598-605.

Frederickson C.J., Klitenick M.A., Manton W.I. and Kirpatrick J.B. (1983). Cytoarquitectonic distribution of Zinc in the hippocampus of man and the rat. Brain Res. 273, 335-339.

Frederickson C.J. (1989). Neurobiology of Zinc and Zinc-containing neurons. I. Rev. Neurobiol. 31, 145-238.

Fujii M. (1967). Topographic peculiarity of the distribution of ^{65}Zn in the brain of intact mice. Bull. Yamaguchi Med. Sch. pp 97-104.

Hassler O. and Soremark R. (1968). Accumulation of Zinc in mouse brain. An autoradiographic study with ^{65}Zn. Arch. Neurol. 19, 117-120.

Jacobs L.S. and Lorenson M.Y. (1986). Cysteamine, Zinc, and Thiols modify detectability of rat pituitary prolactin. A comparison with effects on bovine prolactin suggest differences in hormone storage. Metabolism 35, 209-215.

Judd A.M., Mc Leod R.M. and Login I.S. (1984). Zinc may regulate pituitary prolactin secretion. In: The neurobiology of zinc. Frederickson C.J., Howell G.A. and Kasarskis E.J. (eds). Alan R. Liss Inc. New York. pp 91-104.

Maske H. (1952). Uber den Topochemischen Nachweiss von Zink in Ammonshorn verschiedener Saugetiere. Naturwiss. 42, 424-431.

Mclsaak R.J. (1955). The distribution of ^{65}Zn in rat pancreas. Endocrinology 57, 571-575.

Martinez-Escalera G., Clapp C., Morales M.T., Lorenson M.Y. and Mena F. (1986). Reversal by Thiols of dopamine-, stalk-median eminence-, and zinc-induced inhibition of prolactin transformation in adeno-hypophyses of lacting rats. Endocrinology 118, 1803-1807.

Phil E. (1968). An ultrastructural study of the distribution of heavy metals in the pancreatic islets as revealed by the sulfide silver method. Acta Pathol. Microbiol. Scand. 74, 145-160.

Thorlacius-Ussing O. (1987). Zinc in the anterior pituitary of rat. A histochemical and analytical work. Neuroendocrinology 45, 233-242.

Timm F. (1958a). Zur Histochemie des ammonshorngebietes. Z. Zellforsch. 48, 548-555.

Timm F. (1958b). Zur Histochemie der Schwermetalle. Das sulfidsilberung fahren. Dtsch. Z. Ges. Gerichtl. Med. 46, 706-711.

Vera-Gil A. (1974). La autorradiografia con ^{65}Zn como complemento de la técnica de TIMM para el estudio del Zinc en el substrato endocrino. An. Anat. 23, 141-147.

Accepted December 7, 1993