Zinc in hypothalamus and hypophysis of the rat

C. Pérez-Castejón1, A. Vera-Gil1, M.J. Barra1, M.J. Pérez-Castejón2 and M. Lahoz1

1Department of Morphological Sciences, Faculty of Medicine, University of Zaragoza, Zaragoza and
2Department 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, 65Zn, Histochemistry, Autoradiography

Introduction

The presence of Zn in the central nervous system (CNS) is a fact since it was demonstrated by using intravital dytizone (Maske, 1952; Fleischhauer and Horstman, 1957) and it was confirmed using silver sulphide staining (Timm, 1958a,b). Further evidence was achieved using 65Zn 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 65ZnCl2 named ZAS and supplied by Nuclear Iberica, with a calibrated activity of 355 μCi. 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 autoradiographic 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

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**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 bars 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 intermedia; *: groove 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 adenohypophysial 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


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