SEARCHING FREE ZINC AT THE ULTRASTRUCTURAL LEVEL IN CULTURED ASTROCYTES

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INTRODUCTION

Zinc is necessary for many physiological functions in the body but it plays an important role in diseases provided its balance is altered by environmental, toxicological or idiosyncrasy of subjects. We have focused our investigation in central nervous system, using cultured astrocytes since they are involved in clearance of zinc exocytated to the extracellular medium during synaptic transmission.

MATERIAL AND METHODS

Astrocyte Primary Culture and Alcohol Treatment: cell cultures were prepared from P0 rat fetuses. Cells were cultures in DMEM medium with 5% FCS and were maintained during 7 days at 37ºC with 5% CO_2 in an humidified atmosphere. Ethanol concentration in the treated group was 30 mM.

Zinc Load: cells were loaded with 50 µM $ZnSO_4$ during several times after incubation with TPEN, a zinc chelating agent , to increase both the zinc requirements of cellular and extracellular zinc absorption.

Fluorescence Microscopy: cells were incubated with 50 µg/ml of zinc specific TSQ fluorochrome during 10'.

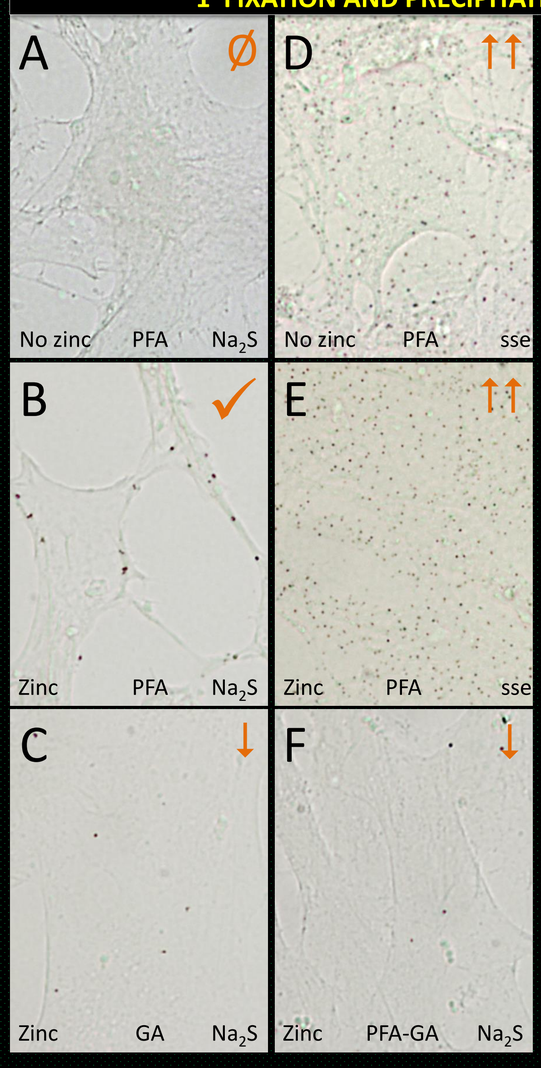
Fixation: cells were fixed with different fixators: 4% PFA, 4% GA and the mix 1% PFA-1% GA.

Zinc Precipitation: two methods were used, 0.1% sodium sulfide in PB with fixed cells during 30 minutes or 5 mM sodium selenite with living cells during 10 minutes.

Transmission Electron Microscopy (TEM): after incubations, cells were fixed, cellular zinc was precipitated, dehydrated and embedded in TAAB resin.

Zinc Autometallography (AMG): both cells monolayer and semithin sections were developed during 30 and 45 minutes respectively at 37ºC in order to obtain specific silver particles over the zinc deposits.

1 FIXATION AND PRECIPITATION METHODS



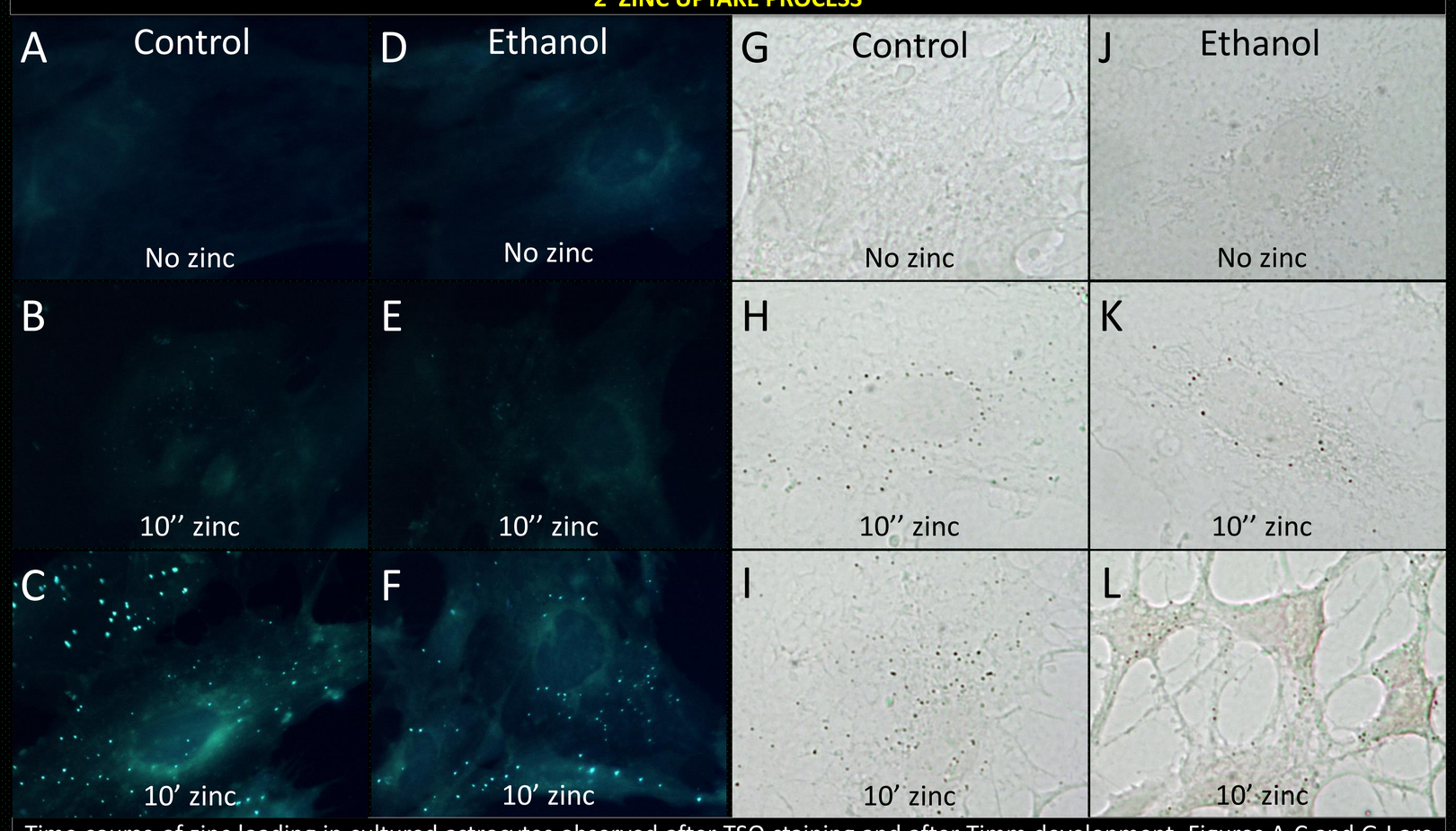
Effect of different fixation and precipitation methods for keeping zinc able to AMG detection.

Astrocytes were fixed with 4% PFA in A, B, D and E; with 4% GA in C; and with the mixture 1% PFA-1% GA in F.

Only PFA fixation previous to Timm development gave us a good mark (B). GA and PFA-GA produced a reduced number of silver particles over zincosomes (C, F).

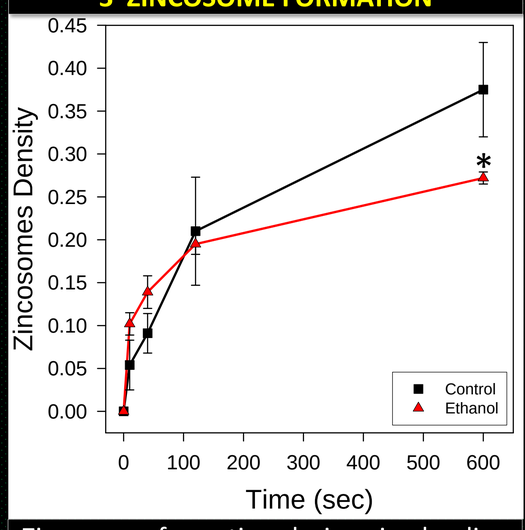
In order to obtain the optimal zinc precipitant for AMG, we used both, sodium selenite (sse) and sodium sulfide (Na_2S). Sodium sulfide was the best choice since sodium selenite gave us false positives in astrocytes without external zinc pulse (D).

2 ZINC UPTAKE PROCESS



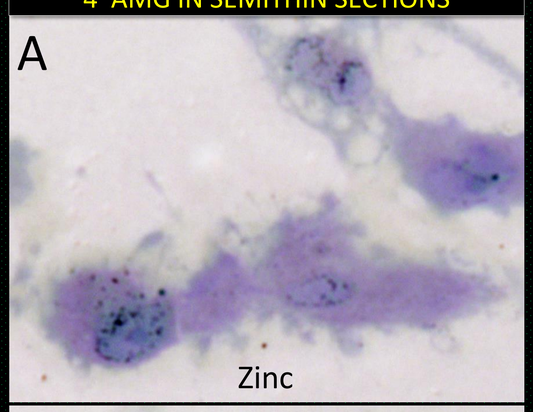
Time course of zinc loading in cultured astrocytes observed after TSQ staining and after Timm development. Figures A-C and G-I are control astrocytes and figures D-F and J-L are astrocytes treated with 30 mM ethanol. We can see that there are no zincosomes with no zinc. Zincosomes already appear at 10 seconds of external zinc pulse. At 10 minutes of incubation it can be seen a reduced density of zincosomes in astrocytes treated with ethanol.

3 ZINCOSOME FORMATION



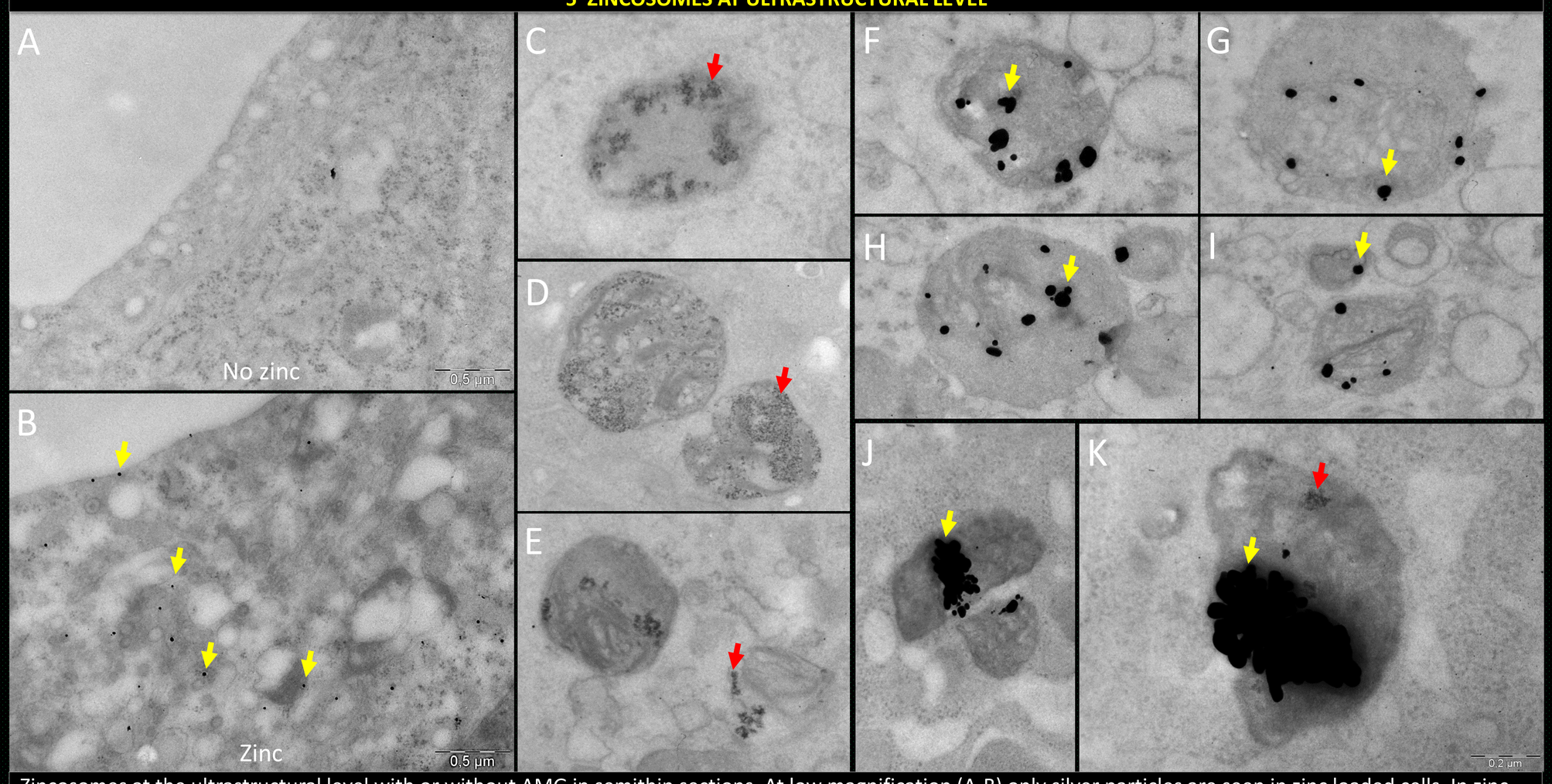
Zincosome formation during zinc loading process. We can see a similar process to form zincosomes in early stages in both control astrocytes and treated with 30 mM ethanol. But at long time of incubation, treated cells shows lower capacity to form zincosomes.

4 AMG IN SEMITHIN SECTIONS



Zinc autometallography in semithin sections. We found that after autometallography astrocytes with external zinc pulse had more silver particles than astrocytes without external zinc.

5 ZINCOSOMES AT ULTRASTRUCTURAL LEVEL



Zincosomes at the ultrastructural level with or without AMG in semithin sections. At low magnification (A-B) only silver particles are seen in zinc loaded cells. In zinc loaded cells and non AMG developed sections (C-E) sulfide-osmium zinc precipitates are observed inside zincosomes. Figures F-K are zincosomes of zinc loaded cells that present silver particles after AMG development. They contain silver particles of different sizes and occasionally both precipitates (sulfide-osmium and silver) can be seen in the same zincosome (K).

CONCLUSIONS

The present study shows that PFA is better than GA and PFA-GA to fix zinc in cultured astrocytes for AMG studies and at ultrastructural level; additionally Na_2S is better precipitator than Na selenite.

Zinc uptake process by astrocytes is very fast, it may be detected in few seconds. In alcohol treated astrocytes the zinc uptake kinetics is similar to control cells in the early stages, but at 10 minutes, alcohol treated astrocytes show less capacity to uptake extracellular zinc to form zincosomes.

At the ultrastructural level, in AMG developed semithins, zincosomes can be easily detected, although its density is lower than that observed in TSQ staining.

More studies are needed to ascertain the differences between these methods: different sensitivity or different zincosome populations?

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