The effect of dietary zinc deficiency on the mossy fiber zinc content of the rat hippocampus

A microbeam PIXE study

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Summary. The effect of dietary zinc deficiency on the mossy fiber zinc content of the rat hippocampus was investigated using PIXE (Particle Induced X-Ray Emission) spectroscopy. Using the proton microbeam (60 x 60 μm), 2 mm line-scans were made on hippocampal sections and the data were expressed as absolute zinc concentrations. Values of 55 and 136 ppm (dry weight) were found for the mean background zinc level and the maximum mossy fiber zinc level, respectively, in animals fed a control diet containing 50 ppm zinc. Treatment of these animals with dithizone caused about 50% reduction in the maximum mossy fiber zinc level. Feeding a zinc-deficient diet for 28 days did not cause a decrease in the mossy fiber zinc level; however, feeding the zinc-deficient diet for 90 days reduced the maximum mossy fiber zinc level by about 30%. The results are discussed in relation to the behavioral abnormalities that have been observed in zinc-deficient animals.

Introduction

The importance of zinc for normal brain development and function has been well documented. Brain growth and maturation are impaired in the offspring of experimental animals as a consequence of zinc deficiency during pregnancy and/or lactation. Such animals exhibit a number of behavioral abnormalities, including learning and memory deficits (Sandstead 1985). Similar behavioral changes can be induced in adult animals by feeding a zinc-deficient diet (Hesse et al. 1979; Gordon et al. 1982); moreover, cerebellar dysfunction and mental changes have been reported in humans made zinc deficient by the administration of excess histidine (Henkin et al. 1975).

Zinc is unevenly distributed in the brain with a notable high concentration in the hippocampus where zinc is found to be associated with the large synaptic boutons of the mossy fiber zone (Haug 1967). A reduction in the hippocampal zinc content by the chelating agent dithizone results in an altered electrophysiological response of the mossy fibers to stimuli (Crawford and Connor 1975). Although this altered response could not be duplicated with the chelating agent diethyldithiocarbamate (Danscher et al. 1975), dietary zinc deficiency was reported to result in a similar alteration of the neuronal transmission through the mossy fibers (Hesse 1979; the zinc content was not measured in this study).

Despite these well-documented neurophysiological alterations that are the result of dietary zinc deficiency, no reductions have been observed in the overall or regional zinc content of the brain, even under conditions of severe zinc deficiency (Wallwork et al. 1983). These studies, however, do not rule out the possibility that zinc deficiency affects primarily the hippocampal mossy fiber zinc concentration, especially when it is considered that this fraction comprises only 8% of the total hippocampal zinc (Frederickson et al. 1983).

Particle Induced X-ray Emission (PIXE) spectroscopy is a sensitive method to measure the concentration of trace element in thin tissue sections. The access to a proton microbeam offers the possibility to study the distribution of trace elements with high spatial resolution. The most important advantage of the microbeam PIXE technique as compared to commonly used histochemical methods to detect trace metals is its ability to provide absolute concentration values which are not influenced by possible masking effects of bulk materials on the trace metals.

We have therefore measured the mossy fiber zinc content in hippocampal sections from rats fed either a zinc-sufficient or a zinc-deficient diet. The results were compared to those of a group of animals that had been treated with dithizone; this method has been reported to reduce the mossy fiber zinc content (Crawford and Connor 1975).

Materials and methods

Experimental animals and diets. Male, 28 days old Wistar rats (Central Institute for the Breeding of Laboratory Animals – TNO, Austerlitz, The Netherlands) were used in this study. The diets were a modification of the IRI-CB purified diet (Van Barneveld and Van den Hamer 1984a). The modification consisted of replacing the casein by ovalbumin as the protein source, omitting methionine and replacing Fe₂O₃ and CuCO₃·Cu(OH)₂ by FeSO₄·7H₂O and CuSO₄·5H₂O, respectively. The control rats (10) were fed this diet supplemented with 50 ppm Zn (as ZnO); a group of 7 animals were fed the diet without the addition of ZnO in which case the Zn content was 0.6 ± 0.2 ppm. The diets and deionized water were provided ad libitum.

Assessment of the zinc status and intravital dithizone treatment. After 14 days on the diets, 5 animals fed the control diet and 5 animals fed the zinc-deficient diet received an intraperitoneal injection of 2 μCi ⁶⁵Zn (0.01 Ci/g of Zn, Radiochemical Centre, Amersham, UK) dissolved in 1 ml of Na-acetate buffer (50 mM, pH 5.6).
tension of the tracer was measured for the following two weeks in a whole-body counting device (Van Barnveld and Van den Hamer 1984b). After 25 days on the diet, the other 5 control animals were injected repeatedly with a solution of dithizone (100 mg in 1 ml ethanol, containing 3 drops of concentrated ammonia and diluted with 9 ml 0.9% NaCl in water). Each animal received 14 intraperitoneal injections (0.2 ml each) over a three day period and a final dose (2 ml) 15 min before the killing of the animals. With the exception of 2 animals that were fed the zinc-deficient diet for a period of 90 days, the other animals were killed after 28 days on the diets.

Zinc content in a number of tissues was determined after wet ashing by atomic absorption spectrometry. Plasma alkaline phosphatase activity was determined using p-nitrophenylphosphate as substrate.

**Preparation of specimen for microbeam PIXE analysis.** Animals were killed by decapitation, the brain was removed and frozen on the quick-freezing element of a cryo-microtome. Hippocampal sections were cut at a thickness of 25 µm. Further processing and the experimental set-up of the proton microbeam were as previously described (Lenglet et al. 1984). The proton beam was programmed to make a linear scan of about 2 mm length in the hilar region of the hippocampus at an approximate right angle to the zinc-rich mossy fiber zone (Fig. 1). The scan was divided into 32 position segments (pixels; 60 µm/position, beam width 60 µm) and an X-ray spectrum was collected from each pixel. Quantitative determinations were performed on the elements K, Zn, Fe and Cu.

**Determination of absolute element concentrations in tissue sections by means of PIXE.** Several methods have been developed to express the experimental data obtained from PIXE measurements in quantitative terms (Bos et al. 1983). We have used the protons scattered from a gold foil as a flux monitor of the proton beam. In thin biological samples, the proton beam loses only a small part of its energy in the sample; the transmitted protons are scattered by a well defined gold foil (100 nm thick) and detected by a surface barrier detector in a fixed geometry (Fig. 2).

The measured number of characteristic X-rays emitted by a trace element Z is given by

\[ N_s = N_p \cdot N_Z \cdot \sigma_Z \cdot \frac{1}{(1 + k_p/k_s)} \cdot \epsilon_Z \cdot \Omega_Z \cdot \epsilon_T \cdot T, \]

where \( N_p \) is the number of incident protons, \( N_Z \) is the number of trace element atoms per cm\(^2\), \( N_A \) Avogadro’s number, \( t_Z \) surface density of element Z (g/cm\(^2\)), \( A_Z \) atomic mass of element Z, \( \sigma_Z \) the K-X-ray production cross-section (cm\(^2\)/sr) averaged over \( \Omega_Z \), \( \epsilon_Z \) the efficiency and solid angle (sr) of the proton detector, respectively. \( \epsilon_T \) a fixed geometry term.

The concentration \( C_Z \) (g/g) of element Z is obtained by dividing the surface density of element Z, \( s_Z \) by the surface density of the surrounding matrix, \( C_{matrix} \). Thus we obtain

\[ C_Z = \frac{N_s}{N_p} \cdot \frac{N_A}{N_Z} \cdot \frac{A_Z}{A_{matrix}} \cdot \frac{\Omega_Z}{\Omega_{matrix}} \cdot \frac{1}{(1 + k_p/k_s)} \cdot \epsilon_T \cdot T_{system} \]

with

\[ K = \frac{\sigma_Z \cdot \epsilon_Z \cdot \Omega_Z \cdot \epsilon_T \cdot T_{system}}{\sigma_p \cdot \epsilon_p \cdot \Omega_p \cdot \epsilon_T \cdot T_{system} + (1 + k_p/k_s)} \]

The value of \( K \) is determined by tabulated values for the cross-sections and the \( k_p/k_s \) ratios, the geometry of the experimental set-up and the specifications of the detectors and windows used.

**Results**

**Assessment of the zinc-deficient status**

Within a few days after starting the feeding of the zinc-deficient diet, the animals became anorexic, they gained very little in weight and lost hair. After 28 days on the diets, the animals were killed and the zinc content in a number of tissues was determined (Table 1). Most notable were the reductions in the zinc content in plasma and bone whereas that in soft tissues like liver, kidney and muscle did not change significantly. Likewise, the zinc content in the brain was not altered. Plasma alkaline phosphatase ac-