Effects of Hyperphenylalaninaemia on the Cerebral Cortex at Ultracellular Levels

There are a few works on the early structural changes in brain due to hyperphenylalaninaemia (HPA) in phenylketonuria (PKU), caused by the absence of the hydroxylase responsible for the phenylalanine-tyrosine step[1]. The cerebral damage must be intense if there is hyperphenylalaninaemia during its development, because there are competitive effects with other amino acids[2] and decreased protein synthesis in experimental models both in vivo and in vitro[3].

However, the results of 4 brain byopsies done for PKU were normal with the photonic microscope[4] with scarce results using electron microscope in experimental rats[5].

We suggest an easy method to describe the intensity of CNS alterations in comparison with controls, referring to ultrastructural findings related with the inhibition of neuronal protein synthesis. We worked with a model used on other occasions to provoke HPA[1,6] and with the electron microscope.

Material. Two litters of 5 white rats were used; 1 control (C) and 1 experimental (E). The mother of the E had a mixture of standard feed and D-L phenylalanine (Phe) to 5% during the first 15 days (beginning a day before delivery). Up to 10% during 60 additional days. The E animals had the same mixed diet from weaning (3 weeks after birth) and up to 15% until the day of sacrifice (at 90 days).

The mothers and the C animals had a standard feed during the complete experiment. All animals (C and E) were killed at 90 days of age.

After an inmediate dissection of the brain ,it was fixed in 2% osmium tetraoxide solution for 30 min at O°C; buffered with sodium cacodilate 0.1 *M* at pH 7.2–7.4. The material was dehydrated in graded acetone, embedded in Araldite, sectioned 600–700 A thick, with III LKB ultramicrotome; contrasts of cuts with lead citrate and examined with a Zeiss EM 9-A.

Electron micrographs ($\times 95{,}000$) were made of neuronal cytoplasm in neurons that completed the following conditions: the same size of nuclei and a visible nucleoli.

The micrographs were divided into arbitrary areas (4 cm^2 each) using a transparent and squared off plate superimpossed on the micrographs (method analized by LOUD et al.[7]). Those areas totally or partially occupied by mitochondrias and cellular membranes were left out. Ribosomal units were counted in a large number of areas to eliminate the differences of ribosomal distribution in the neuron cytoplasm.

Discussion. The decresed number of ribosomes in experimental neurons reflect an alteration in their development and differentiation, since the overload of Phe was given during the animals' growing period.

The lower number of ribosomes in experimentals, as seen by us with the electron microscope, demonstrates an inhibition of neuronal protein synthesis. Ribosomes have a direct relation with the quantity and complexity of the synthetized protein molecules[8,9]. This inhibition could be explained by the high levels of Phe in blood and tissue which alter transportation of other amino acids to the CNS[2]. Besides, phenylalanine is the amino acid with the greatest capacity for disaggregating polyribosomes in brain[3].

In conclusion, we studied with the electron microscope the ultrastructure of CNS in PKU animals and found, using an easy method, a significant decrese of ribosomes in the experimentals but not in the controls.

Results.

Animal	Sex	Weight (g)	N areas (4 cm^2)	N ribosomes	*X* ribos/area
E1	♂	85	394	2217	5.60
E2	♂	75	215	1012	4.70
E3	♂	85	214	1671	7.80
E4	♀	67	259	1832	7.07
E5	♀	92	324	2199	6.78
C1	♂	100	315	3254	10.33
C2	♂	100	264	2767	10.48
C3	♂	95	338	3397	10.05
C4	♀	100	390	3467	8.88
C5	♀	107	197	2125	10.78

Average of ribosomes by neuronal areas (*X*) in 4 cm^2 of an electron micrlgraph of CNS (parietal cortex). Magnification: $\times$ 95,000. Control animals (C) compared to hyperphenylalaninemics (E) since birth, treated for 90 days with overload of pure phenylalanine.

Age (days)	Treatment	N	*X* rib/area ($\pm$ S.E.M.)	Control (%)	*P*
90	Control	5	10.10 ± 0.32		
90	Phenylalanine	5	6.39 ± 0.55	63	< 0.001

Student's *t*-test was used to value the significance of the average between controls and experimentals.

Resumen. La alteración de la síntesis protéica en el SNC originada por los niveles altos de fenilalanina en sangre se refleja en una disminución de imágenes ribosomales en el citoplasma neuronal.

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