SUBCELLULAR STUDY OF THE PGLU-PEPTIDASE I ACTIVITY DURING THE RAT BRAIN DEVELOPMENT

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There are several peptides (thyroliberin, luliberin, neurotensine,etc) whose N-terminal ending is a pyroglutamic residue (pGlu). This cyclated glutamic acid allows them to have more resistance to the degradation by aminopeptidases, the most active proteolytic enzymes of the bain. However, nowadays, it has been characterized two aminopeptidases capable of hydrolyzing this N-terminal pGlu: pyroglutamyl-peptidase II (O'Connor and O'Cuinn, 1985; Wilk and Wilk, 1989), with high specificity of thyrotrophin releasing hormone (TRH), and pyroglutamyl-peptidase I (Browne and O'Cuinn, 1983), which can degradate the majority of pGlu-peptides. This last enzyme has been described in the cytosol and in the particulate fraction (Alba et al., 1995).

Changes of some of the pyroglutamyl-containing peptide levels have been reported during the first postnatal month in several brain regions (Gayo et al., 1986; Lamberton et al., 1984) and these modifications have been related to changes in the pGlu-peptidase activity (Fuse et al., 1990; Vargas et al. 1992; Gandarias et al. 1994).

The role of the pGlu-peptidase II in the synaptic degradation of TRH is quite clear (O'Cuinn et al. 1990). However, the physiological function of pGlu-peptidase I remains obscure.

In order to ascertain the possible role of the pyroglutamyl-peptidase I on the pGlu-peptide levels modulation in the developmental rat brain, the present work studies this enzyme activity during the first postnatal month in several subcellular fractions of the brain subcortex.

Male Sprague-Dawley rats, maintained under controlled conditions, were used in this investigation.

The ages of the animals were embryonic day 22 (E22), parturition day (P0) and postnatal days 2 (P2), 4 (P4), 6 (P6), 9 (P9), 14 (P14), 20 (P20), 25 (P25) and 30 (P30).

The animals were set under Equithensin anaesthesia (2 ml/kg body wt) and perfused with saline plus 50mM of phosphate buffer solution (pH 7.4).

Subcortex was taken by dissection and homogenized in 10 volumes of 0.32 M sucrose.

Subcellular fractions under study (cytosolic, nuclear, mitochondrial, microsomal, synaptosomal and myelinic fractions) were obtained according to the method of Gray and Whittaker (1962), modified by Krueger et al. (1977).

The purity of the synaptosomal fraction has been checked by Electronic Microscopy.

Briefly, from the crude mitochondrial pellet (12,500 g), we obtained fractions A (myelinic), B (synaptosomal) and C (mitochondrial). Fractions B, C, and the microsomal pellet (100,000 g) and samples from the crude nuclear pellet (1000 g), were homogenized in Tris-HCl 10 mM (pH 7.4) and centrifuged (100,000 g, 30 min, 4°C). Samples from these supernatants and those previously obtained at 100,000 g (cytosol) were used to detect soluble activity and proteins, assayed per triplicate. The resultant pellets, washed three times to avoid contamination of the soluble enzyme, and fraction A (myelin) were homogenized in Tris-HCl 10 mM (pH 7.4), plus 1% of Triton X-100, to obtain, after centrifugation (100,000 g, 30 min, 4°C), supernatants which were employed to detect membrane-bound activity and proteins also in triplicate.

PGlu-peptidase activity was fluorimetrically measured by a modification (Alba et al., 1989) in the method of Greenberg (1962), using pGlu-β-naphthylamide as substrate and DTT and EDTA as activators of pGlu-peptidase I and inhibitors of pGlu-peptidase II.

The utilization of this fluorogenic substrate allows us to measure aminopeptidase activity without using more expensive and less safe radioisotopic techniques.

One unit of pGlu-peptidase activity (UAP) is the amount of enzyme that hydrolyzes one picomole of pGlu-β-naphthylamide per minute.

Protein concentration was measured in triplicate by the method of Bradford (1976).

The results were analyzed by the analysis of the variance (ANOVA test) and in those cases where we found significant changes, the comparison between means was done by Fisher´s PLSD test.

The developmental patterns of the soluble and the membrane-bound pGlu-peptidase I activities, obtained in this research, in the synaptosomal fraction show that the soluble activity remains low until P9 and significantly increases from P9 to P14 (Fisher´s PLSD test $p<0.01$). These high levels of activity do not change significantly from P14 to P30. The membrane-bound activity seems not to have any significant increase except two peaks at P2 (Fisher´s PLSD test $p<0.01$) and P25 (Fisher´s PLSD test $p<0.05$).

On the other hand, significant decreases in the soluble pGlu-peptidase I activity were observed in the cytosolic fraction (ANOVA test $p=0.001$),where activity declines more than 65% during the first postnatal month, in the nuclear fraction (ANOVA test $p=0.001$), with a decrease of 50% during the same period, and in the microsomal fraction (ANOVA test $p=0.002$),with high levels of activity from E22 to P6 and lower levels after P9. However we did not find significant changes in the mitochondrial fraction (ANOVA test $p=0.806$). The membrane-bound activity did not show significant changes in the myelinic (ANOVA test: $p=0.179$), microsomal (ANOVA test: $p=0.158$) and nuclear (ANOVA test: $p=0.257$) fractions but there is a peak at P2 (Fisher´s PLSD test $p<0.01$) in the mitochondrial fraction.

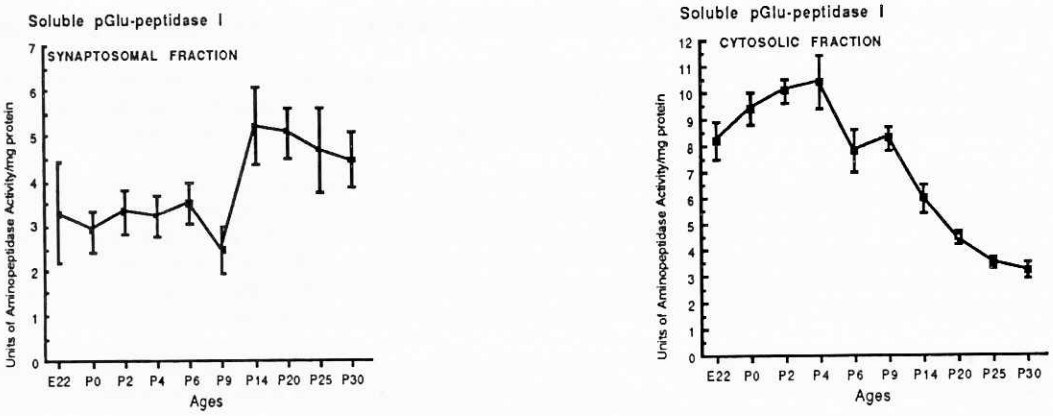

Figure 1.- Soluble pGlu-peptidase I activity during the first postnatal month in the synaptosomal fraction and in the cytosol. It can be observed that the developmental patterns are distinct in both different subcellular structures. Values, recorded as UAP/mg protein, represent mean±SEM (n=10).

In summary, in this work we have found age-related changes in pyroglutamyl-peptidase I activity in several subcellular fractions of the rat brain subcortex. The profiles of the developmental changes are different depending on the studied subcellular fraction (fig. 1) and the changes in the membrane-bound activities are quite different and generally less drastic than in the soluble form. It is suggested that this enzyme could have different physiological roles in the distinct subcellular structures during the brain development.

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