

DEVELOPMENTAL CHANGES OF TYR-AMINOPEPTIDASE ACTIVITY IN DIFFERENT SUBCELLULAR FRACTIONS OF THE SUBCORTICAL REGION OF THE RAT BRAIN.

Adolfo VARONA, Javier GIL, Nerea SAITUA, Mónica GALLEGO and Jon IRAZUSTA,
 Department of Physiology, Medical School, University of the Basque Country. P.O. Box 699. Bilbao, Spain.

Endogenous opioids seem to play important roles in the regulation of the development of the nervous system (Hauser et al., 1987). Opioid peptides have been described as natural trophic factors in brain development and several studies have reported opioid-induced alterations in neural growth. Enkephalins, the first endogenous opioid peptides isolated, participate in the regulation of growth in the developing rat nervous system (Zagon et al., 1994) and also serves as a growth factor (Zagon et al., 1993). It is known that the effects of enkephalins are of short duration, as a result of their hydrolysis by brain enzymes. A major pathway of enkephalin degradation occurs via the cleavage of the Tyr-Gly amide bond by aminopeptidases (Thorsett and Wivrat, 1987). Three aminopeptidases have high affinity for enkephalins (Hersh, 1982); one soluble (Dyer et al., 1990) and two membrane-bound, puromycin-sensitive (or aminopeptidase MII) and puromycin-insensitive (or aminopeptidase M) (Giros et al., 1985). Nowadays, it is accepted that this proteolytic group is involved in the maturation of the rat brain (Gandarias et al., 1989). The establishment of the subcellular localization of enkephalin-degrading enzymes and their eventual changes during the development are important for understanding the regulatory mechanism controlling the activity of enkephalins.

Male Sprague-Dawley rats were used in this investigation. The ages of the animals were embryonic day 22, parturition day (P0) and postnatal days 2, 4 and 6. The animals were perfused under Equithensin anaesthesia with saline (pH 7.4). Brain subcortices were taken by dissection and homogenized in 10 volumes of 0.32 M sucrose. Subcellular fractions were obtained according to the method of Gray and Whittaker (1962), modified by Krueger et al. (1977). Briefly, from the crude mitochondrial pellet (12,500 g), we obtained fractions A (myelin), B (synaptosomal) and C (mitochondrial). Fractions B, C, and microsomal pellet (100,000 g) and samples from 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. The resultant pellets 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. Tyr-aminopeptidase activity was fluorimetrically measured by a modification in the method of Greenberg (1962), using Tyr- β -naphthylamide as substrate. The membrane-bound activity was detected entirely and after incubating with 20 μ M puromycin. Protein concentration was measured by the method of Bradford (1976). The results were analyzed by the analysis of the variance and the comparison between means was done by Fisher's test.

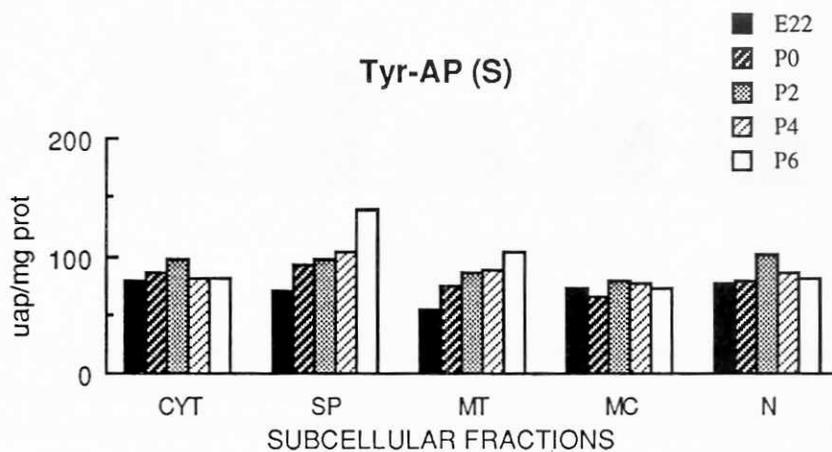


FIGURE 1

Soluble Tyr-aminopeptidase activity in the subcellular fractions under study is given in fig 1. In the synaptosomal (SP) and mitochondrial (MT) fractions, Tyr-aminopeptidase increase significantly during the first postnatal week. However there is no significant changes in the nuclear (N), microsomal (MC) and cytosolic (CYT) fractions.

Age-related changes of the membrane-bound and puromycin-sensitive aminopeptidase activity are given in fig 2. In the synaptosomal and mitochondrial fractions, this enzyme behaves very similarly to soluble activity. Both increase during the first postnatal week. In the myelinic (MY) fraction, we have also found age-related increases during the first postnatal week. In the microsomal and nuclear fractions, the aminopeptidase M-II activity peaks at P2, with lower levels during the rest of the week.

Figure 3 shows the developmental changes of the puromycin-insensitive membrane-bound aminopeptidase activity. In general, the patterns of the variations are different from puromycin-sensitive activities. In the synaptosomal, myelinic and mitochondrial fractions, the activity increase from E22 to P2 and afterwards decreases until the end of the first week. In the

nuclear fraction the activity also increases from the birth. In the microsomal fraction the activity remains without changes until p6, when decreases abruptly.

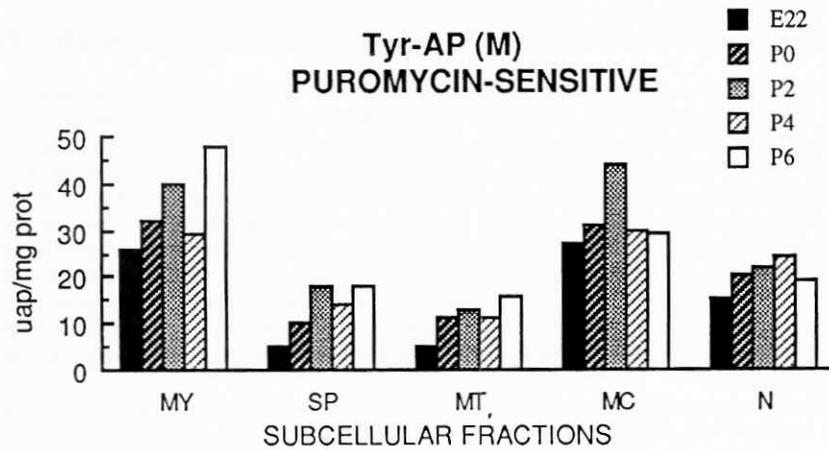


FIGURE 2

These results show to us that enkephalin-degrading aminopeptidases are developmentally regulated. The most significant increases of aminopeptidase activity occurs during the first postnatal week (while the period of axonal and dendritic growth) are produced in the synaptosomal fraction. So, it is suggested that the enkephalin-degradation at the synapsis could play an important role in the axonal and dendritic growth.

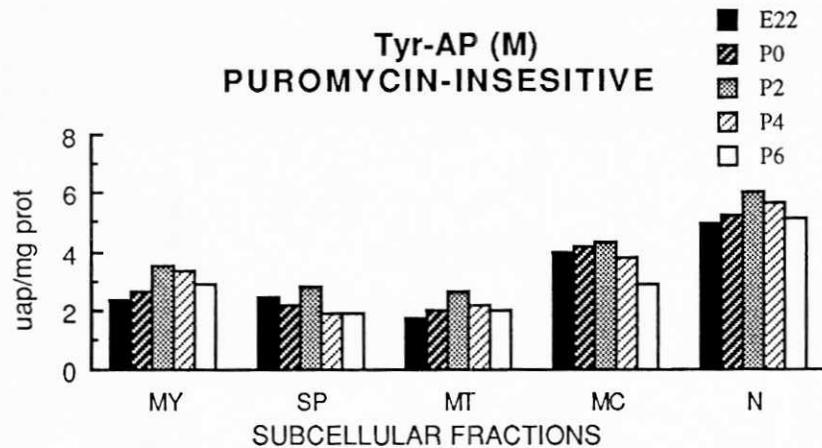


FIGURE 3

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