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Short Contribution

Pyroglutamil-peptidase I activity in the cortex of the cat brain during development

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ABSTRACT Thyrotropin Releasing Hormone (TRH) is a principal regulator of thyroid system function. However, significant concentrations of TRH were found throughout the central nervous system, the cortex being one of the areas most richly endowed with thyroliberin. Research concerning the functional role of this brain peptide is performed, in part, by studying peptidase enzymes which may be involved in the inactivation of the peptide. The pGlu-His bond is cleaved by two pyroGlu-peptidases: I (soluble) and II (membrane-bound). In the present investigation, developmental activity of the soluble form is described in the cortices of the cat brain. The selected maturation stages were 15 and 30 days postnatal. The cortices were the frontal, parietal, area 17 and areas 18 and 19 as a whole, distinguishing brain hemispheres in all cases. PyroGlu-aminopeptidase I activity increased significantly with age in all the brain regions except area 17. It is suggested that this enzyme activity plays a part in the neurochemical changes that take place during brain maturation.

KEY WORDS: *pyroglutamyl-aminopeptidase, thyroliberin, thyroliberin-degrading enzyme, brain development, cat brain*

The structure of thyrotropin releasing hormone (TRH or thyroliberin) was first elucidated in 1969 and was reported to be a tripeptide with the amino acid sequence pyroGlu-His-ProNH_2 (Boler *et al.*, 1969; Burgus *et al.*, 1969). This peptide stimulates the release of pituitary TSH (thyrotropin), following attachment to high affinity receptors on the thyrotrope. The highest concentration of TRH was found to be present in the hypothalamus. However, significant concentrations of TRH are found throughout the central nervous system. Although such concentrations are small when compared with the hypothalamic levels, quantitatively over 70% of total brain TRH is found outside this region. The extrahypothalamic areas most richly endowed with TRH are the cerebral cortex, the thalamus, the brain stem and the spinal cord (Leppaluoto *et al.*, 1978). This extrahypothalamic distribution of the tripeptide suggests that, in addition to its neuroendocrine role, it may also function as neurotransmitter or neuromodulator in the CNS.

Alterations in TRH physiology seem to occur during the perinatal period in the developing brain. Fetal and neonatal plasma of rats, sheep and humans contains relatively high TRH concentrations (Mudge and Fellows, 1973; Lambardi *et al.*, 1978) and low or undetectable TRH degrading enzyme activities (Aratan-Spire *et al.*, 1983). In the rat brain, TRH values gradually increase to adult levels by the end of the fourth postnatal week. However, the mechanisms for the change in brain tissue thyroliberin levels during development are not clear. Thus, different developmental profiles for enzymes controlling TRH activity have been described in rat (Fuse *et al.*, 1990) and mouse brain (Faivre-Bauman *et al.*, 1981). In the present study, we addressed possible changes in TRH metabolism in the cortex of the cat brain. The selection of the cat to carry out this research was due to the fact that this species is phylogenetically more developed than the mouse or the rat, and information concerning pyroGlu-aminopeptidase activity in this animal is lacking (this not being the case with rat and mouse). The selection of the cortices as experimental tissue was for be one of the extrahypothalamic area most richly endowed with TRH.

The pGlu-His bond is cleaved by two pyroglutamyl-peptidases: pGlu-aminopeptidase I (soluble) and pGlu-aminopeptidase II (membrane-bound) (Bauer and Kleinkauf, 1980; Bauer *et al.*, 1981; McDonald and Barrett, 1986). In the present paper, developmental activity of the soluble form is described.

Fig. 1 (upper and lower) shows the pGlu-aminopeptidase I activity levels of 15 (n=9) and 30 (n=9) days postnatal in all the cortical areas under study. Pyroglutamyl-aminopeptidase activity increased significantly with age in the frontal (right and left) cortex ($p<0.005$), parietal (right and left) cortex ($p<0.005$) and in the areas 18 and 19 as a whole in the right ($p<0.005$) and left ($p<0.05$) hemispheres. No

Abbreviations used in this paper. TRH, Thyrotropin Releasing Hormone or thyroliberin; pGlu-AP, pyroglutamyl-aminopeptidase; pGlu-NA, pyroglutamyl-2-naphthylamide; NA, 2-naphthylamine.

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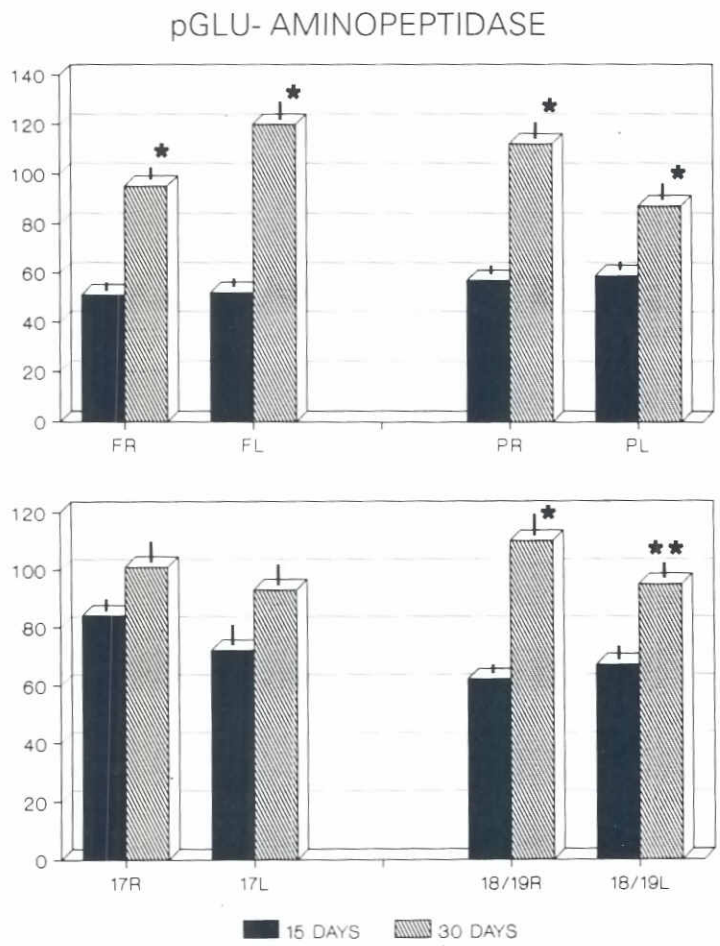

Fig. 1. Age-related changes of pyroglutamyl-aminopeptidase I activity in the left and right (L, R) frontal (F) and parietal (P) cortices, *area 17 (17) and areas 18 and 19 as a whole (18/19). The ordinate scale represents the enzyme activity in units of aminopeptidase/mg protein. The abscissa scale represent the different brain regions of the cat assayed in this research Aminopeptidase activity was fluorimetrically measured in triplicate using pGlu-2-naphthylamide as substrate. The results are expressed as units of aminopeptidase/mg protein. Each column is the mean for nine animals, with the standard error. (*p<0.005; **p<0.05).*

significant differences between the two stages were observed in area 17. However, in this region, no significant increases were appreciated from 15 to 30 days postnatal. On the other hand, no significant differences were detected when comparing the cortices with each other.

Over the past 15 years, evidence has accumulated indicating that certain peptides that were known to exert biological effects outside the central nervous system may also possess neurotransmitter or neuromodulator functions in brain (Kow and Pfaff, 1988). Attention has also been paid to the peptidases of brain that may be involved in the inactivation of these peptides, and several review articles have previously appeared detailing the progress made in the study of these enzymes (Griffiths and McDermott, 1984; O'Cuinn *et al.*, 1990). Information concerning developmental alterations in these peptidases has also been presented by several groups (Kato *et al.*, 1979; Prasad *et al.*, 1983; Gandarias *et al.*, 1989a; Gandarias *et al.*, 1989b; Gandarias *et al.*, 1989c).

The results obtained in this research show, first, that all the cortical areas under study have pGlu-aminopeptidase I activity during both developmental stages assayed. The distribution and levels of this enzyme in the brain are currently receiving special attention as a result of the recognition of several neurological functions for thyroliberin and its metabolites. As endogenous pyroGlu-aminopeptidase I (pGlu-AP I) activity is responsible, at least in part, for the rapid inactivation of TRH by brain extracts (Bauer and Kleinkauf, 1980), it is suggested that the cortices of the cat brain have the potential of contributing to the degradative control of thyroliberin. Since lesions of the thyrotropic area or deafferentation (Jackson and Lechan, 1983) of the rat hypothalamus leaves the extrahypothalamic brain contents of TRH undisturbed, it appears that TRH in the extrahypothalamic nervous system is not derived from the hypothalamus and that «*in situ*» synthesis occurs. This fact, together with the results reported here, could reinforce the possibility that thyroliberin possesses a neurotransmitter or neuromodulator role in the CNS.

We also reported here on the changes of specific activity of pGlu-AP I during the development of the cat brain cortices. All age differences in the present study consisted of a higher enzyme activity for 1-month stage than for that found in 15-day-old cats. These changes could reflect decreases in the activity of TRH during cortical maturation, with the decrease depending on how much TRH is synthesized. In any case, the action of soluble pGlu-AP I on TRH produces His-ProNH_2, and this metabolite has been reported to cyclize spontaneously and nonenzymatically to produce His-Pro diketopiperazine (DKP), at neutral and alkaline pH (Peterkofsky *et al.*, 1982; Prasad *et al.*, 1982). His-Pro diketopiperazine, which does not appear to be further degraded by any enzymatic mechanism, is itself reported to possess endocrine activity (Brabant *et al.*, 1981; Melmed *et al.*, 1982) as well as numerous central nervous actions (Peterkofsky *et al.*, 1982; Prasad *et al.*, 1982). Thus, increases in the pGlu-AP activity possibly reflect increases in the formation of DKP.

At least in the mouse brain, when the pyroGlu-aminopeptidase I has a higher activity, TRH is just detectable (Faivre-Bauman *et al.*, 1978), and these levels coincide with the first postnatal stages. Our results seem to coincide with those obtained by Faivre-Bauman *et al.* (1981) in the mouse brain, who showed increases in this enzyme activity from birth until 13th day of life. Then it decreases to adult values. However, in the rat brain, Fuse *et al.* (1990) show a decrease in this activity from the 3rd day postnatal. Thus, a considerable controversy exists concerning the possible changes in TRH-degrading enzymes during brain maturation. Nevertheless, at least in the rat brain, TRH levels gradually increase up to adult values, but only from the end of the fourth postnatal week. Therefore, in the cortices of the cat brain, TRH levels could be reduced until the precise developmental period, and these levels could be regulated, at least in part, by this enzyme activity. Also changes in the cyclic(His-Pro) (DKP) levels could occur.

Experimental Procedures

Eighteen cats were studied in these experiments. The ages of the animals were 15 days (n=9) and 30 days (n=9) postnatal. In order to avoid proteolytic contamination from the serum, animals from two groups were

perfused with saline plus 50 mM phosphate buffer, pH 7.4, through the left cardiac ventricle under Equithensin anesthesia. The brains were quickly removed and cooled in dry ice. Brain samples, taken by dissection, were as follows: Frontal Cortex (Right and Left), Parietal Cortex (R & L), Area 17 (R & L) and Areas 18 and 19 as a whole (R & L). The brain samples were homogenized (in Tris HCl 10 mM, pH 7.4) and ultracentrifuged (100,000 g, 35 min) to obtain the soluble fraction. The resulting supernatant was used for the analysis of pyroglutamate-aminopeptidase I activity and proteins. All preparative steps were carried out at 4°C.

PyroGlu-aminopeptidase activity was fluorimetrically measured in triplicate using pyroglutamyl-2-naphthylamide (pGlu-NA) as substrate, by the method of Greenberg (1962) with the recent modifications of Alba *et al.* (1989), but using 50 mM Tris HCl, pH 7.4 instead of 50 mM phosphate buffer solution (Bauer *et al.*, 1981): 10 µl aliquots of soluble fraction were incubated with 1 ml of pGlu-NA (1 mg/100 ml) in serum albumin (10 mg/100 ml) and dithiothreitol (10 mg/100 ml) in 50 mM Tris HCl buffer, pH 7.4. The reaction was stopped by the addition of 1 ml of 0.1 M acetate buffer solution, pH 4.2. The 2-naphthylamine (NA) released was determined by measuring the fluorescent intensity at 412 nm with excitation at 345 nm. Relative fluorescence was converted to femtomols of NA by comparison with a standard curve. Protein concentration was measured in triplicate by the method of Bradford (1976). The results were recorded as units of pGlu-aminopeptidase I activity per milligram of protein (mean ± SEM). One unit of aminopeptidase activity is the amount of enzyme that hydrolyzes one femtomol of pGlu-NA per minute. Differences between means were calculated using Student's *t* test.

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