BRAIN PYROGLUTAMATE-PEPTIDASE I ACTIVITY DEVELOPS DIFFERENTLY IN HYPOTHALAMUS AND PITUITARY GLAND OF THE FEMALE AND MALE RATS.

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Two enzymes for cleaving the N-terminal pyroglutamate (pGlu) of several peptides have been described (O´Cuinn et al., 1990). pGlu-peptidase I, cleaves all pGlu-amino acid bonds except pGlu-Pro. Substrates of pGlu-peptidase I, in addition to TRH, include LH-RH, neurotensin and bombesin. pGlu-peptidase II is a membrane-bound enzyme and appears to be a specific TRH degrading enzyme.

The first postnatal month of the rat, marked by dramatic changes in neuropeptide physiology, including those with N-terminal pGlu, offered a suitable model for studying the role of pGlu-peptidase. Information concerning developmental alterations in the neuropeptides (Lamberton et al., 1984; Wray & Hoffman, 1986) and their peptidases (Gandarias et al., 1992; Vargas et al., 1992) has been presented by several groups. However, information regarding the brain distribution and ontogeny of pGlu-peptidase I activity is fragmentary and controversial. Thus, different developmental profiles have been described in the rat hypothalamus (Aratan-Spire et al., 1983; Fuse et al., 1990). Moreover, previous ontogenetic studies of pGlu-peptidase were performed using a pool of animals, without differentiating for sex.

In the present work, we addressed possible changes in the ontogeny of pGlu-peptidase I activity in the hypothalamus and the pituitary gland of the male and the female rat. We have made a sexual differentiation because previous studies described sexual differences in the physiology of peptides with N-terminal pGlu during the first postnatal month (Gayo et al., 1986; Wray and Hoffman, 1986 a, b).

Male and female Sprague-Dawley rats were used in this investigation. The ages of the animals were 9, 12, 15, 20 and 25 days postnatal. Animals from five groups were perfused with saline plus 50 mM phosphate buffer, pH 7.4 under equithensin anaesthesia. The brains were removed to disect the hypothalamus. Hypothalami and pituitary glands were homogenized (in Tris HCl 10 mmol/l, pH 7.4) and ultracentrifuged (100,000 g, 35 min) to obtain from the supernatant the soluble fraction.

pGlu-peptidase I activity was fluorometrically measured in triplicate using pGlu-β-naphtylamide as sustrate, by a modification of the method of Greenberg (1962). Protein concentration was measured in triplicate by the method of Bradford (1976). The results were recorded as units of pGlu-peptidase I activity per mg of protein (mean$\pm$ standard error of the mean). One unit of peptidase activity is the amount of enzyme that hydrolyzes 1 picomol of pGlu-β-naphtylamide per minute. Significance of the sexual differences was confirmed with Student´s t-test. For multiple comparison, ANOVA was followed by Fisher´s PLSD test.

Figure 1 shows the pGlu-peptidase I activity levels of 9, 12, 15, 20 and 25 days postnatal in the hypothalamus and the pituitary gland of male and female rats. In the hypothalamus, pGlu-peptidase activity decreases from 9 to 15 days after birth. Sharply between day 9 and day 12 and steadily from day 12 to days 15 or 20 day (depending on sex). After, the enzyme activity does not show any significant changes. At all ages under study, the pGlu-peptidase activity is greater in the males than in the females. There are no significant age-related changes in the pituitary gland. However, as in the hypothalamus, significant sex dependent differences are detectable

Results obtained in this research show that the hypothalamus and the pituitary gland, have lower specific activity in females. Several groups have observed significant sexually dependent differences in the number of LH-RH neurons (Wray & Hoffman, 1986 a,b) and the hypothalamic content of TRH (Gayo et al., 1986) during the first month of postnatal life.

On the other hand, earlier ontogenetic studies have reported changes in pGlu-peptidase I activity in several brain areas using a pool of male and female rats (Aratan-Spireet al., 1983; Fuse et al., 1990). These studies have shown different developmental profiles for pGlu-peptidase I. Thus, while some of the reports have demonstrated decreases in the activity of the enzyme in the cerebral cortex and the hypothalamus from the 7th until the 20th postnatal day, others described constant enzyme activity during the first twenty days of life.

Although the role of soluble peptidases is controversial, some observations suggest a role for pGlu-peptidase I in regulating intracellular levels of TRH (O´Cuinn et al., 1990). Previous reports have described that the brain content of thyroliberin increased up to day 35 (Nemeskeri et al., 1985), and, at least in the hypothalamus, steadily until day 20 and dramatically between days 21 and 23 (Aratan-Spire et al., 1983). Changes in the activity of pGlu-peptidase I are not necessarilly related to TRH, since other peptides such as LHRH and neurotensin are also substrates for this enzyme. However, it might be reasonable to suggest that the decrease observed in brain pGlu-peptidase I up to day 20 may well be related to the simultaneous increase in thyroliberin concentration. Moreover, Taylor et al. (1990) have found that hypothalamic TRH mRNA content remained unchanged from the 3rd to the 21st postnatal day. The slight decrease in the enzyme activity between day 20 and 25 cannot explain the dramatic rise of TRH between days 21 and 23. This suggests that an increase in gene transcription, translation or altered posttranslational events are likely to contribute to elevated TRH levels during this period.

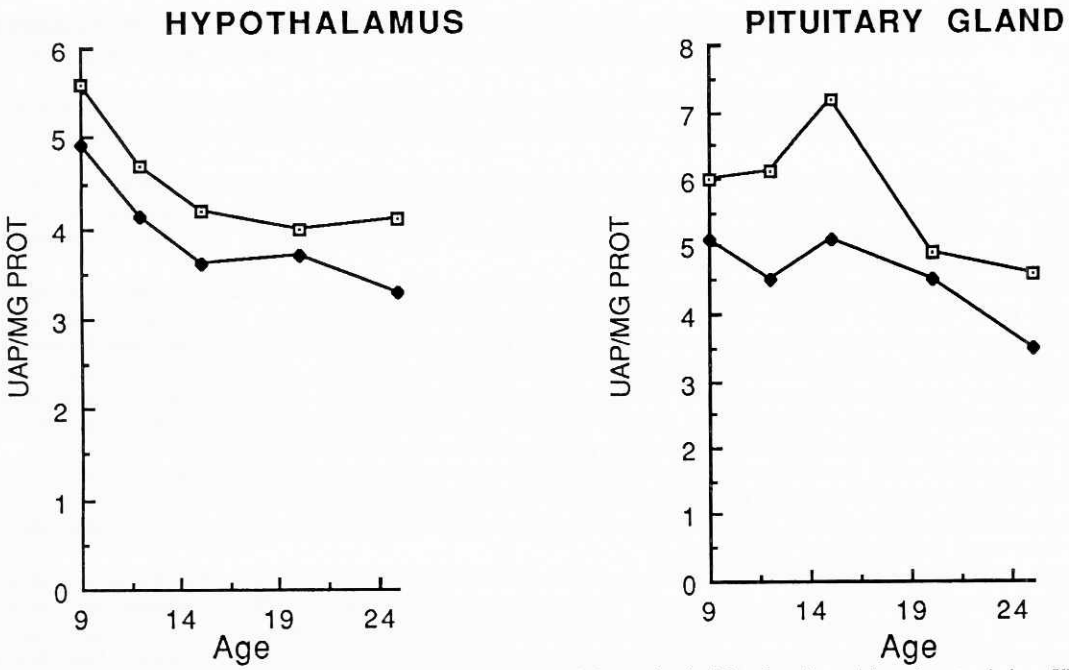

Figure 1.- PGlu-peptidase I activity levels in the hypothalamus (Ht) and the pituitary gland (PG) of male and female rats during different stages of development (9, 12, 15, 20, 25 = postnatal days). Values, recorded as units of peptidase activity per mg of protein, represent mean± SEM.

In summary, this study demonstrated developmental changes in hypothalamus and pituitary gland pGlu-peptidase I activity in both sexes. In addition, we have described sex-related differences. Then, it could be suggested that this enzyme might play a part in the normal development of the female and male rat brain.

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