Serum and Brain Aminopeptidase Activities in Cyclic Rats

J. M. De Gandarias, M. Ramirez, E. Echevarria, J. Irazusta and L. Casis

Department of Physiology, Medical School, University of Pais Vasco, P. O. Box 699, Bilbao, Spain

Abstract. Research into the functional role of brain peptides is performed, in part, by studying peptidase enzymes which might be involved in the processing or inactivation of the brain peptides. Aminopeptidase activity has been proposed as a candidate for the regulation of the degradation of these peptides. In this paper, acid (Asp-) and basic (Arg-) aminopeptidase activities were studied in several brain regions and in the serum during the estrous cycle of the rat. Asp-aminopeptidase activity did not significantly change at any point. However, a marked rise was found in Arg-aminopeptidase activity in all the brain areas studied and the serum during the proestrus. It is suggested that this activity plays a role in the hormonal changes that take place during the cycle, possibly in regulating the activity of several neuroactive peptides.

Key words: Aminopeptidase activity — Rat serum — Rat brain — Estrual cycle

Introduction

The aminopeptidases (AP) are proteolytic enzymes found in virtually all mammalian cells. In the brain, these enzymes have been suggested as the possible mechanism of the regulation and biotransformation of several neuropeptides (Hayashi 1978; Hersh and McKelvy 1981). The regulation of the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland is one of the major interest in reproductive endocrinology. A host of neuropeptides affecting gonadotropin secretion has recently been described. There is a abundance of evidence favouring a predominant tonic inhibitory role of endogenous opioid peptides and neuropeptide Y in the regulation of LH secretion (Allen et al. 1987; Berglund et al. 1988). Functional activities of neuropeptides can be investigated by studying their degradation under the influence of exopeptidases. Some of these proteolytic enzymes (the AP mentioned above), along with hydrolyzing neuropeptides, are also capable of hydrolyzing chromogenic substrates of the aminoacyl-2-naphthylamides type.

To throw some light on the possible function of AP *in vivo*, it seems appropiate to measure the serum and brain activities in experimental animals during the estrous cycle. Cyclic alterations in neutral AP activity have recently been described (de Gandarias et al. 1988). The present report extends this observation to the cyclic activities of basic and acid AP, by measuring the hydrolysis rates of the substrates Arg-2-naphthylamide (Arg-2-NA) and Asp-2-naphthylamide (Asp-2-NA) (basic and acid substrates respectively).

Materials and Methods

Adult female Sprague-Dawley rats were used in this investigation. The animals were kept under controlled illumination (light interval from 07:00 to 19:00) and had unlimited access to water and standard rat chow. Timing of the estrous cycle was determined by examining daily vaginal smears and only rats showing two or more regular cycles were selected for the experiments. The animals were grouped into estrus, diestrus and proestrus groups ($n = 8$, each). All the rats used were 3 months; this is important in view of age-related changes of AP activities from 2 weeks to 3 months (de Gandarias et al. 1989c), without further changes in older animals (de Gandarias et al. 1989a). The animals were set under Equithensin anesthesia (2 ml kg body wt) and perfused with saline plus 50 mmol l phosphate buffer, pH 7.4, from the left cardiac ventricle. Blood samples were taken from the left cardiac ventricle before perfusion and immediately centrifuged to obtain serum (S), which was used as the source of enzymes and protein. The brains were quickly removed and cooled on dry ice. Pituitary gland (Pg) and samples taken by dissection from the frontal (F), parieto-temporal (PT) and occipital (O) cortices and hypothalamus (Ht) were homogenized (in 10 mmol l Tris HCl, pH 7.4) and ultracentrifuged (100,000 × *g*, 35 min) to obtain te soluble fraction. The resulting supernatant was used for the analysis of AP activities and proteins. All preparative steps were carried out at 4 °C.

Aminopeptidase activities were measured fluorimetrically (412 nm with excitation at 345 nm) in parallel triplicate samples using Arg- and ASP-2-NA as substrates, according to methods described previously (Alba et al. 1986; de Gandarias et al. 1989a, c). Protein concentration was measured in parellel triplicate samples by the method of Bradford (1976).

The results were expressed as AP units per milligram of protein (mean ± SEM). One AP unit is the amount of the enzyme that hydrolyzes one picomol aminoacyl-2-naphthylamide per minute (or per eighteen hours in the case of ASP-2-NA). Differences between mean values were analyzed by Student's *t*-test, and the groups were compared using analysis of variance (ANOVA).

Results

The values obtained using Asp-2-NA for the brain areas and the serum during the estrus stage were: 13.6 ± 2.4 F; 11.0 ± 1.6 PT; 11.3 ± 1.3 O; 26.4 ± 4.7 Ht; 20.9 ± 2.3 PG; 2.46 ± 0.16 S. There were no significant differences between the results at this stage and those obtained for the rest of the cycle (diestrus and proestrus) (except for a no significant decrease in the Ht and PG in the proestrus). On the other hand, higher activity levels were found for Ht and PG;

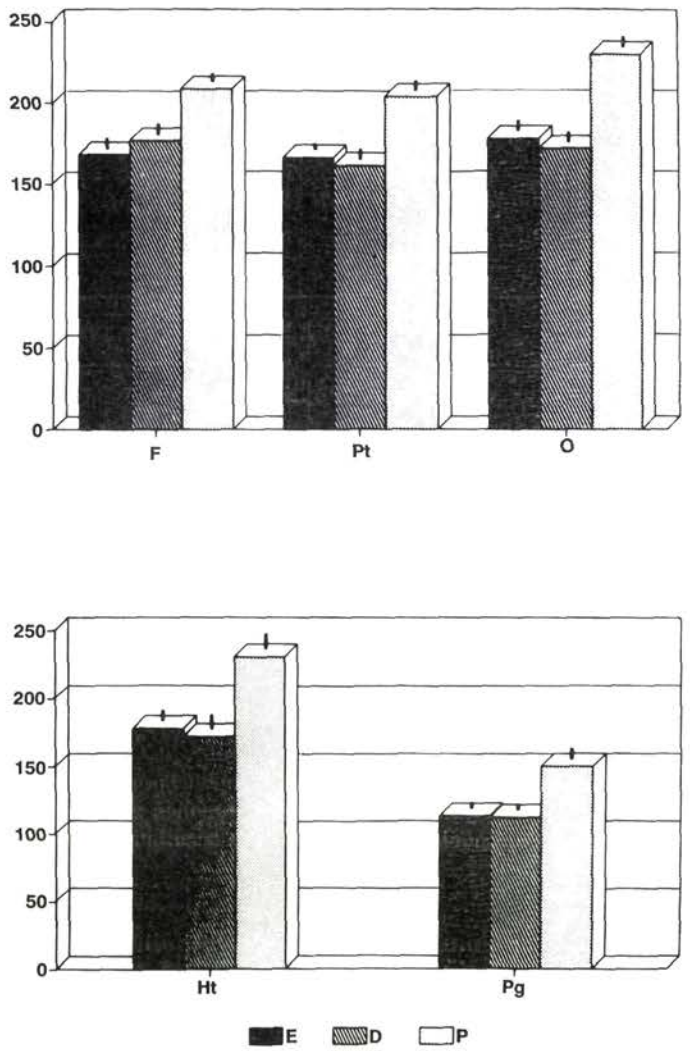

Fig. 1. Arg-aminopeptidase activity at estrus (E), diestrus (D) and proestrus (P) in the cortex regions studied (frontal: F; parieto-temporal: PT, occipital: O), hypothalamus (Ht) and pituitary gland (Pg). Values represent mean ± SEM (units of aminopeptidase/mg protein).

this is in agreement with previous results (de Gandarias et al. 1989a). The results obtained in the brain areas using Arg-2-NA as substrate are shown in Fig. 1. The serum results were: 0.22 ± 0.04 estrus; 0.18 ± 0.03 diestrus; 0.35 ± 0.05 proestrus.

The basic AP activity showed a significant increase ($p < 0.05$) at proestrus in all the brain areas under study and in the serum, but the activity was unchanged during estrus and diestrus. The results concerning regional distribution of Arg-AP activity are also in agreement with previous reports (de Gandarias et al. 1989a, b, c).

Discussion

The results reported here reveal marked variations in Arg-AP activity during the rat estrual cycle. The pattern is similar to that observed with gonadotropin secretion, in particular LH. Although the specific function of the AP in the estrous cycle remains unknown, these enzymes seem to be involved in the regulation of the release dynamies of gonadotropins. This is suggested by the similar development of both groups of agents during the estrual cycle, and also by their similar qualitative changes after orchidectomy and ovariectomy (de Gandarias et al. 1989b). However, since the cyclic variation of AP ativity resembles to that of gonadotropin (with a peak in the proestrus), it is conceivable that the enzyme action is not directed entirely at the metabolic control (time-rate limiting) of gonadotropins or their hypothalamic releasing factors. It may therefore be reasonable to suggest that neutral (de Gandarias et al. 1988) and basic (Arg-AP) aminopeptidases could be implicated in the above processes since, as pointed out in Introduction, several neuropeptides have recently been proposed that could regulate the release of gonadotropins by inhibiting the hypothalamic releasing factors (Allen et al. 1987; Berglund et al. 1988). It is difficult, within the frame of this hypothesis, to speculate about the changes observed in the different cortex regions. However, it has been reported recently that gonadotropin hormones releasing cells project to a number of brain areas, including the cortex (Zoeller et al. 1988). Therefore, a more specific analysis of the relationship of sexual hormones to brain peptides and to *in vivo* brain enzyme activity might be of interest.

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