Lys- and Tyr-arylamidase activities in serum and brain during the estrous cycle of the rat

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Abstract. Peptidase enzymes are involved in neuropeptide processing or degradation. In order to analyse a possible functional participation of these enzymes, arylamidase activity of several rat brain regions and serum was assayed in the soluble fraction during the estrous cycle using L-Lys and L-Tyr-ß-naphthylamide as substrates. Significant differences were present in the hypothalamus, the pituitary and serum when both substrates were used. However, there were no differences in cortical areas. These results suggest a role for arylamidase activity in the hormonal changes that happen during the estrous cycle of the rat.

Neuropeptide degrading enzymes evidently play a role in processing and inactivation of hypothalamic and hypophyseal hormones (1). Arylamidases are among such enzymes, the activities of which can be measured by analysing the hydrolysis of artificial substrates like aminoacyl-ß-naphthylamides (aa-ß-NA; arylamides). Although arylamidases have been implicated in the degradation of several neuropeptides (2), their functional role in the brain is still unknown. The possible endogenous substrates could be neuropeptides containing the same amino acid at the N-terminal position as arylamides, in this case Lys or Tyr.

The hypothalamic-pituitary axis plays a major role integrating endocrine, autonomic and behavioural responses of animals to various internal and external stimuli. The cyclic hormonal changes during the reproductive cycle enable us to establish a comparative study with the enzymatic activity probably implicated in their regulation.

We report here a study analysing the activity obtained with Lys. and Tyr-ß-Na as substrates during the estrous cycle in serum, the hypothalamus, the pituitary gland and three cortical regions of adult female rats.

Materials and Methods

Adult female albino rats (300–350 g) were grouped at estrus, diestrus and proestrus (N = 8 each) verified by vaginal smear (3). In order to remove plasmatic arylamidases, their brains were perfused from the left cardiac ventricle with saline plus 50 mmol/l of phosphate buffer (pH 7.4) under equithesin anesthesia (2 ml/kg) this included: 42.5 g/l of chloralhydrate, 0.1621 of Nembutal® 0.3961 of propylene glycol and 21.3 g/l of magnesium sulphate in distilled water. Blood samples were obtained before perfusion from the left cardiac ventricle and centrifuged to obtain serum which was used as an enzyme and protein source. The brains were quickly removed (less than 60 sec) and cooled in dry ice. Pituitary gland and tissue samples from frontal, parietotemporal, occipital cortex and the hypothalamus were homogenized in 10 mmol/l of TRIS HCl (pH 7.4) and ultracentrifuged (100 000 × g, 35 min, 4°C) in order to obtain the soluble fraction. The resulting supernatant was decanted and used for the determination of arylamidase activity and proteins.

Lys- and Tyr-arylamidase activities (Lys-AR and Tyr-AR) were measured, in triplicate, using Lys- and Tyr-ß-NA as substrates according to the modified method of Greenberg (4): 10 μl of supernatant were incubated at 37°C in a substrate solution that contained: 8 mg/l of Lys- or Tyr-ß-NA, 100 mg/l of bovine serum albumin, and 100 mg/l of dithiothreitol in 50 mmol/l of phosphate buffer (pH 7.4). The reaction was stopped by the addition of 1 ml of 0.1 mol/l of acetate buffer (pH 7.2). The β-naphthy-
lamine released was determined fluorimetrically at 412 nm of emission with an excitation of 345 nm. Results were expressed as units of arylamidase activity per mg of protein. One unit was defined as the amount of enzyme that hydrolyzes 1 pmol of Lys- or Tyr-β-NA per min at 37°C. Proteins were measured, in triplicate, by the method of Bradford (5).

Comparison between groups (more than two means) were evaluated by the ANOVA test and differences between two means by the Student's *t*-test.

Results and Discussion

The ANOVA test showed significant differences between groups (estrus, diestrus and proestrus) in serum, the hypothalamus and the pituitary gland (but not in the cortex) using both Lys- and Tyr-β-NA as substrate.

The results are presented in Fig. 1 (Lys-AR) and Fig. 2 (Tyr-AR). Levels of Lys-AR activity were higher than Tyr-AR in all structures and serum.
There was no significant changes in Lys-AR and Tyr-AR (Figs. 1A and 2A) in the frontal, parieto-temporal and occipital cortex at different stages of the estrous cycle.

In serum, neither activities changed between estrus and diestrus. At proestrus however, Lys-AR showed a significant increase, from estrus (p < 0.05) and diestrus (p < 0.001) (Fig. 1D) and Tyr-AR also was significantly higher at proestrus (p < 0.01) than at estrus (Fig. 2D).

In the hypothalamus and pituitary, Lys-AR (Fig. 1B and 1C, respectively) and Tyr-AR (Fig. 2B and 2C, respectively) reached significant maximal levels at proestrus as compared with estrus (p < 0.01), Lys-AR also showed significant differences between diestrus and proestrus in the hypothalamus (p < 0.05) and pituitary (p < 0.01), but the activity did not change between estrus and diestrus.

From these results we cannot draw any functional meaning for the involved enzymes but the evidence that these activities are being significantly modified during the estrous cycle in the hypothalamus, the pituitary and serum, suggests a direct participation, probably linked to the hormonal changes that occur at these levels during the ovarian cycle. In this way, an implication of gonadal steroids could be postulated; they induce ovulation during the estrous cycle, influence the secretion of pituitary hormones such as prolactin, a follicle-stimulating and luteinizing hormone and besides, are involved in several brain functions including sexual behaviour, monoamine turnover, increase or decrease in enzymatic activity or stimulation of the synthesis of proteins and RNA (6). Finally, according to this hypothesis, estrogen-concentrating neurons in the brain of the female rat are present essentially in the hypothalamus but not at cortical levels (7).

In conclusion, this study demonstrates that: a. Lys- and Tyr-arylamidase activities (resulting from the use of Lys- and Tyr-β-naphthylamide as substrates) show significant changes in serum, the hypothalamus and pituitary during the rat estrous cycle; and b. there are no significant changes in the frontal, parieto-temporal and occipital cortex during the rat estrous cycle (when both substrates are used).

References