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Short Contribution

Pre- and postnatal aminopeptidase activities in the rat brain

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ABSTRACT Research concerning the functional role of brain peptides is performed, in part, by studying peptidase enzymes which might be involved in brain peptide processing or inactivation. Aminopeptidase (AP) activity has been proposed as a candidate regulator of the degradation of these peptides. In this paper, changes in Lys- and Leu-aminopeptidase activities in rat brain hemispheres, cerebellum and medulla were examined in 20 day fetuses and one day postnatal subjects. Aminopeptidase activities were studied by measuring the rate of hydrolysis of the artificial substrates Lys- and Leu-2-naphthylamides (fluorimetrically detected in triplicate). Both enzyme activities increase from the last fetal stage up to the first day of birth in all the brain areas examined except for the case of Leu-AP activity in the medulla. It is suggested that these activities play a part in the neurochemical changes that take place during rat brain maturation, possibly by regulating the activity of several neuroactive peptides.

KEY WORDS: *aminopeptidase activity, brain development, cerebral hemispheres, cerebellum, medulla*

Protein turnover is highly active in the brain, and most proteins are in a dynamic state. Although changes in proteolytic enzymes in developing rat brain have been reported by several groups (Hui and Lajtha, 1978; Kato *et al.*, 1979; Faivre-Bauman *et al.*, 1981), the physiological role of the peptidases is still unknown. It has been suggested that some of these enzymes (the aminopeptidases) could regulate the levels of several neuropeptides (NP), such as enkephalins and endorphins (Hayashi, 1978; Hersh and McKelvy, 1981), angiotensin II (Kelly *et al.*, 1983) and substance P (Benuck and Marks, 1975). In our laboratory the physiological role of aminopeptidases (AP) has been studied in several brain regions during the estrual cycle of female rats (de Gandarias *et al.*, 1988, 1989a; Casis *et al.*, 1989). It has been suggested that AP activities could be related with the surge of gonadotropin.

Recently we have also described some developmental alterations in AP activities, showing changes from two week to three month periods (de Gandarias *et al.*, 1989d), without further modifications in older animals (de Gandarias *et al.*, 1989b). It has been suggested that these changes could be related to some of the processes of brain maturation. To complete our present study, in this paper we report the presence and the changes, during pre- and postnatal stages of rat brain, of Lys- and Leu-AP activities. These proteolytic enzymes, as well as hydrolyzing NP, are also capable of hydrolyzing chromogenic substrates of the aminoacyl-2-naphthylamides type, first introduced by Gomori (1954). The study was performed by measuring the rate of hydrolysis of the substrates Leu-2-naphthylamide (Leu-NA) and Lys-2-naphthylamide (Lys-NA), by neutral and basic aminopeptidase activities respectively.

Fig. 1 (upper) shows the Lys-AP activity levels of embryo (20 days) (n=12) and neonatal (1 day) (n=12) male rats (not female because some sexual differences in AP activities have been recently described) (de Gandarias *et al.*, 1989c). In all structures, activity with Lys-NA showed significant increase with age. The neurochemical changes were found to be higher in the cerebral hemispheres ($p < 0.001$). The other regions showed a comparable developmental profile but with different statistical significance: $p< 0.005$ for cerebellum and $p < 0.05$ for medulla.

Fig. 1 (lower) shows the Leu-AP activity levels at the same stages of Lys-AP. The activity also developed postpartum. There is a significant increase in the right ($p<0.005$) and left hemisphere ($p < 0.01$) and in the cerebellum

Abbreviations used in this paper: AP, aminopeptidase; Leu-NA, Leu-2-naphthylamide; Lys-NA, Lys-2-naphthylamide; NA, naphthylamine; NP, neuropeptide.

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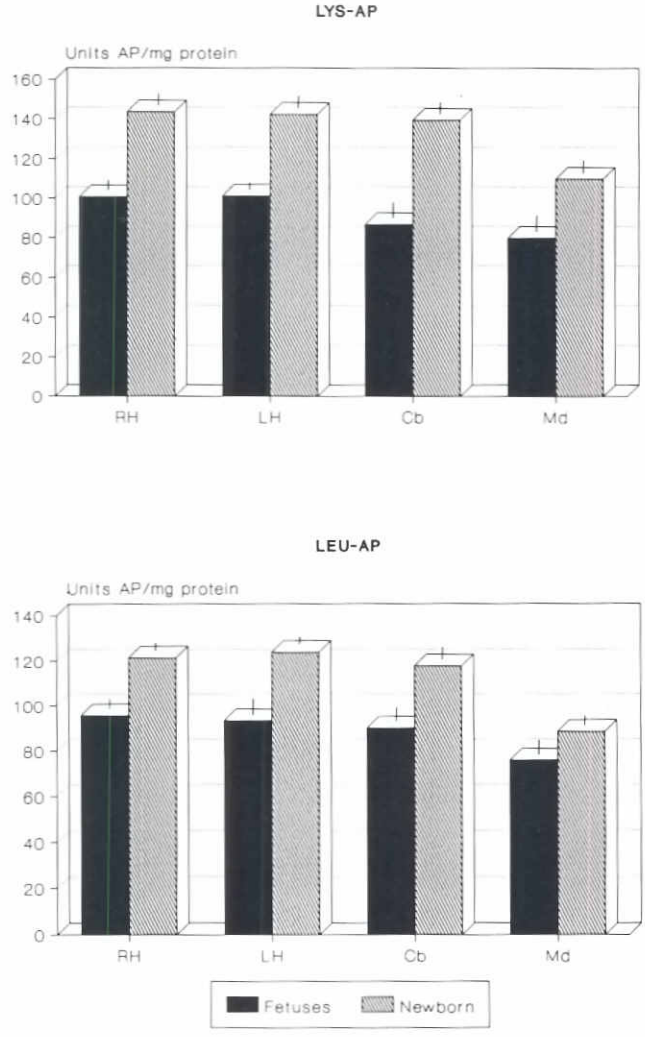

Fig. 1. Brain aminopeptidase activity. *Enzimatic activity of Lys- (LYS-AP) and Leu-aminopeptidase (LEU-AP) in discrete brain regions (RH: right hemisphere; LH: left hemisphere; Cb: cerebellum; Md: medulla) of fetuses (n=12) and newborn (n=12) male rats. Vertical lines denote the standard error.*

($p<0.05$), but the activity was constant in the medulla when both stages were compared.

The aim of this report is to communicate the changes in activity of two neuropeptide degrading enzymes, the Lys- and Leu-AP, during rat brain development. This activity increases from the last fetal stage up to the first day of birth in both brain hemispheres, cerebellum and medulla, except for Leu-AP activity in the medulla. Recent studies report that AP activities increase progressively during postnatal stages (de Gandarias *et al.*, 1989d) and that they reach maximum levels at two months postnatal. This is followed by a decline until three months, after which the activities are maintained without further changes in older animals (de Gandarias *et al.*, 1989b). These results suggest that AP activities may be related to the development of the nervous system.

However, because we have no knowledge, even during adulthood, of the morphological site of brain peptidases and of their relationship to the sites of peptide breakdown, and because the involvement of peptidases in the NP catabolism is still relatively unknown, it would be premature to draw any precise conclusion on the possible role of such AP in controlling the level of available peptides. The increase observed in AP activity could be related to a decrease in the activity of diverse neuropeptides. Recent studies have observed that the concentration of several NP is high at the embryonic stage and declines in adulthood (Bartolomé *et al.*, 1986; Hayashi, 1987). These changes are possibly related to the electrical inactivity in the cerebral tissue during the embryonic stage and/or to the possible actuation of the NP as a growth regulatory factor. To support this view, it has been recently suggested that these neuropeptides (enkephalins, angiotensin II, neuropeptide Y, etc.) are also involved in the control of hormonal pituitary secretion (Allen *et al.*, 1987; Berglund *et al.*, 1988). Due to the fact that AP (Lys-, Leu-, Arg- and Tyr-AP) change their activity in contrast to what happens to NP during the estrous cycle (de Gandarias *et al.*, 1988; 1989a; Casis *et al.*, 1989;), it could be the case that these proteolytic enzymes play a part in the regulation of these NP.

Experimental procedures

Male Sprague-Dawley rats, at 20 days of embryonic (n=12) and one day postnatal (n=12) development were used in this research. Animals were killed by decapitation between 9 and 10 a.m., and brains were quickly removed and rinsed twice in phosphate buffer saline (pH 7.4) to remove blood.

Brain samples were rapidly removed from the cerebral hemispheres, cerebellum and medulla. Samples were homogenized in 10 vol. of 10 mM Tris HCl buffer, pH 7.4. The homogenate was centrifuged at 100,000 xg for 35 min and the supernatant aspirated and used as the enzyme and protein source. All preparatory steps were carried out at 4 °C.

Brain aminopeptidase activities were fluorimetrically measured in triplicate using Lys- and Leu-NA as substrates (Sigma Chem. Co., St. Louis, Mo.) by the method of Greenberg (1962) with a slight modification (Alba *et al.*, 1986): 10 µl aliquots of soluble fraction were incubated for 30 min with 1 ml of either Leu-NA (0.8 mg/dl) or Lys-NA (0.9 mg/dl), bovine serum albumin (10 mg/dl) and Dithiothreitol (10 mg/dl) in 50 mM phosphate 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 picomoles of NA by comparison with a standard curve (NA was purchased from Sigma). Results were expressed as units of AP per mg protein (mean ± SEM). One unit of AP activity was defined as the amount of enzyme that hydrolyzes 1 pmol of Leu-NA or Lys-NA per minute, at 37°C. Protein concentration was measured in triplicate by the method of Bradford (1976).

Statistical analysis was performed by the Student's t test.

References

ALBA, F., RAMIREZ, M., IRIBAR, C., CANTALEJO, E. and OSORIO, C. (1986). Asymmetrical distribution of aminopeptidase activity in the cortex of rat brain. *Brain Res. 368*: 158-160.

ALLEN, L.G., CROWLEY, W.R. and KALRA, S.P. (1987). Interactions between

neuropeptide Y and adrenergic systems in the stimulation of luteinizing hormone release in steroid-primed ovariectomized rats. *Endocrinology 121*: 1953-1959.

BARTOLOMÉ, J.V., BARTOLOMÉ, M.B., DALTNER, L.A., EVANS, C.J., BARCHAS, J.D., KUHN, C.M. and SCHANBERG, S.M. (1986). Effect of ß-endophin on ornithine decarboxylase in tissues of developing rats: a potential role for this endogenous neuropeptide in the regulation of tissue growth. *Life Sci. 38:* 2355-2362.

BENUCK, M. and MARKS, N. (1975). Enzymatic inactivation of substance P by a partially purified enzyme from rat brain. *Biochem. Biophys. Res. Commun. 65* : 153-160.

BERGLUND, L.A., DERENDORF, H. and SIMPKINS, S.P. (1988). Desensitization of brain opiate receptors mechanism by gonadal steroid treatment that stimulate luteinizing hormone secretion. *Endocrinology 122:* 2718-2726.

BRADFORD, M.M. (1976). A rapid and sensitive method for the quantitation of microgram of protein utilizing the principle of protein-dye binding. *Anal. Biochem. 72:* 248-254.

CASIS, L., ECHEVARRIA, E., IRAZUSTA, J., MUGICA, J. and CASIS, L. (1989). Brain Asp- and Arg-aminopeptidase activity levels in cyclic rats. *Eur. J. Neurosci. 2 (Suppl.)*: 221.

FAIVRE-BAUMAN, A., KNISTSCHECK, H., TIXIER-VIDAL, A. and BAUER, K. (1981). Ontogenesis of neuropeptide degrading enzymes in the mouse brain. *J. Neurosci. Res. 6*: 63-74.

GANDARIAS, J.M. DE, CASIS, L., IRAZUSTA, J., ECHEVARRIA, E. and RAMIREZ, M. (1988). Changes of aminopeptidase activity levels in serum and brain during the estrous cycle of the rat. *Horm. Metab. Res. 20*: 776.

GANDARIAS, J.M. DE, CASIS, L., IRAZUSTA, J., ECHEVARRIA, E., ARECHAGA, G. and RAMIREZ, M. (1989a). Lys- and Tyr-arylamidase activities in serum and brain during the estrous cycle of the rat. *Acta Endocrinol. 121*: 671-673.

GANDARIAS, J.M. DE, CASIS, L., MUGICA, R., ARECHAGA, G. and RAMIREZ, M. (1989b). Aging and brain aminopeptidase activity. *Rev. Esp. Fisiol. 45:* 89-94.

GANDARIAS, J.M. DE, RAMIREZ, M., ZULAICA, J. and CASIS, L. (1989c). Aminopeptidase (arylamidase) activity in discrete areas of the rat brain: sex differences. *Horm. Metab. Res. 21*: 285-286.

GANDARIAS, J.M. DE, RAMIREZ, M., ZULAICA, J., IRIBAR, C. and CASIS, L. (1989d). Postnatal development of aminopeptidase (arylamidase) activity in rat brain. *J. Biochem. 105:* 44-46.

GOMORI, G. (1954). Chromogenic substrates for aminopeptidase. *Proc. Soc. Exp. Biol. Med. 87:* 559-561.

GREENBERG, L.J. (1962). Fluorimetric measurement of alkaline phosphatase and aminopeptidase activities in the order of 10^{-14} mole. *Biochem. Biophys. Res. Commun. 9:* 430-435.

HAYASHI, M. (1978). Monkey brain arylamidase. II. Further characterization and studies on mode of hydrolysis of physiological active peptides. *J. Biochem. 84:* 1363-1372.

HAYASHI, M. (1987). Ontogeny of glutamic acid decarboxylase, tyrosine hydroxylase, choline acetyltransferase, somatostatin and substance P in monkey cerebellum. *Develop. Brain Res. 32:* 181-186.

HERSH, C.B. and McKELVY, J.F. (1981). An aminopeptidase from bovine brain which catalyzes the hydrolysis of enkephalin. *J. Neurochem. 36:* 171-178.

HUI, K.S. and LAJTHA, A. (1978). Prolidase activity in rat brain; developmental, regional and subcellular distribution. *Brain Res. 153:* 79-85.

KATO, T., HAMA, T., NAGATSU, T., KUZUYA, H. and SAKAKIBARA, S. (1979).Changes of X-prolyl-dipeptidyl-aminopeptidase activity in developing rat brain. *Experientia 35:* 1329-1330.

KELLY, J.A., NEIDLE, E.L. and NEIDLE, A. (1983). An aminopeptidase from mouse brain cytosol that cleaves N-terminal acidic aminoacid residue. *J. Neurochem. 40:* 1727-1733.

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