Int. J. Dev. Biol. 36: 583-585 (1992)

Short Contribution

Aminopeptidase activity levels during axonal and dendritic growth in the rat brain

JUAN M. DE GANDARIAS, JON IRAZUSTA, ENRIQUE ECHEVARRÍA, ELENA SAN MARTÍN and LUIS CASIS*

Department of Physiology, Medical School, University of the Basque Country, Leioa, Spain

ABSTRACT **Brain aminopeptidase activity has been suggested as a candidate for the regulation and biotransformation of several neuropeptides. In this paper, changes in Lys- and Leu-aminopeptidase activities in rat brain hemispheres, cerebellum and medulla were examined in 1-, 3-, 5- and 7-days postnatal subjects. Aminopeptidase activities were studied by measuring the rate of hydrolysis of the artificial chromogenic substrates Lys- and Leu-2-naphthylamides (fluorimetrically detected in triplicate). Both enzyme activities show a decrease on the 3rd day of life followed by increases on the 5th and 7th day postbirth. It is suggested that these activities could play a part in the neurochemical changes that take place during axonal and dendritic growth in the rat brain.**

KEY WORDS: *Leu-Aminopeptidase, Lys-Aminopeptidase, postnatal development, rat brain*

The aminopeptidases (AP) are proteolytic enzymes found in virtually all mammalian cells. In the central nervous system, these enzymes have been suggested as the possible mechanism responsible for the regulation and biotransformation of several neuropeptides (NP) (Turzynski and Mentlein, 1990). In the last few years, different patterns of aminopeptidase activity in developing rat brain have been reported by several groups (Faivre-Bauman *et al.*, 1981; Gandarias *et al.*, 1989a,b,c). The changes described until now were increases from 2 week to 1-2 month periods, without further modifications in older animals (Gandarias *et al.*, 1989a,c). Recently, it has also been demonstrated that there are several increases in these proteolytic activities immediately after birth (Gandarias *et al.*, 1989b). It has been suggested therefore that the developmental changes in arylamidase activities could be related to some of the processes of brain maturation. Nevertheless, the critical developmental period of the rat brain includes four well-defined ontogenic periods: (a) cell proliferation, prior to birth, (b) axonal and dendritic growth, during the first postnatal week, (c) myelination, up to 25 days and (d) the slow deposition of solids in young adults. As was pointed out, during the 1st, 3rd and 4th phases of the critical period, several changes in AP activities have been demonstrated. However, possible changes in AP during axonal and dendritic growth have not been described. Thus, the present report completes previous observations, describing the developmental changes of Lys- and Leu-aminopeptidase activities during the first week of life in different structures of the rat brain. The study was performed by measuring the rate of hydrolysis of the substrates Leu- and Lys-2-naphthylamide, by neutral and basic aminopeptidase activities respectively. The brain structures under study were the right and left hemispheres, the cerebellum and the medulla.

Analysis of variance, for both kinds of enzyme activities, revealed several significant differences with relation to the selected ages ($p<0.05$). Fig. 1 shows the evolution of Lys-aminopeptidase activity in the selected brain areas. In all structures, the enzyme activity showed significant changes with age: significant decreases from 1st to 3rd day ($p<0.001$) and increases on day 5 ($p<0.01$ for RH, Cb and Md, and $p<0.001$ for LH) and day 7 postbirth ($p<0.05$ RH and $p<0.005$ LH). No significant differences in the cerebellum and the medulla were observed between the 5th and the 7th days.

Fig. 2 shows the Leu-aminopeptidase activity levels at the same stages of Lys-AP. This second assayed activity also showed alterations postpartum. Thus, there is a significant decrease on the 3rd day in the right and left ($p<0.001$) hemispheres, cerebellum ($p<0.001$) and medulla ($p<0.05$). After this stage, the activity showed (in both hemispheres) an increase on the 5th ($p<0.005$) and 7th days ($p<0.001$). In the cerebellum and the medulla, significant increases from 3rd to 5th day were observed ($p<0.001$). However, no differences between the 5th and 7th stages in these structures were detected. On the other hand, Lys-AP activity was always higher than Leu-AP activity in all structures ($p<0.01$). No significant differences between cerebral hemispheres in any of the developmental stages under study were observed.

Although changes in proteolytic enzymes in developing rat brain have been reported by several authors (Faivre-Bauman *et al.*, 1981;

Abbreviations used in this paper: AP, aminopeptidase; Leu-NA, Leu-2-naphthylamide, Lys-NA, Lys-2-naphthylamide; NA, 2-naphthylamine; NP, neuropeptides; RH, right hemisphere; LH, left hemisphere; Cb, cerebellum; Md, medulla.

***Address for reprints**: Department of Physiology, University of the Basque Country, P.O. Box 699, E-48080 Bilbao, Spain. FAX: 34-4-464.81.52.

0214-6282/92/$03.00

Printed in Spain

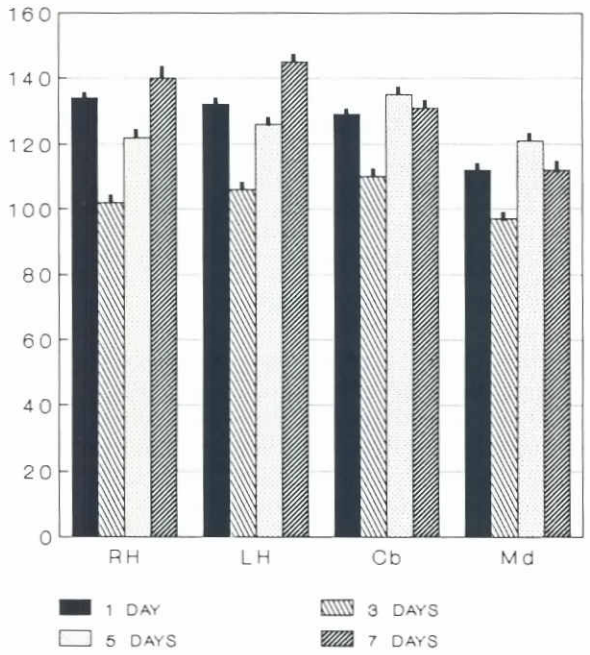

Fig. 1. Age-related changes of Lys-aminopeptidase activity in the right (RH) and left (LH) hemispheres, cerebellum (Cb) and medulla (Md). *Aminopeptidase activity was fluorimetrically measured in triplicate using Lys-2-naphthylamide as substrate. The results are expressed as units of aminopeptidase/mg protein. Each column is the mean for 10 animals, with the standard error.*

Gandarias *et al.*, 1989a,b,c), the physiological role of the peptidases still remains relatively unknown. It has been suggested that some of these enzymes (the aminopeptidases) could regulate the levels of several neuropeptides. Thus, the conversion of Met-Lys-bradikinin and Lys-bradikinin to bradikinin (Camargo *et al.*, 1972), the hydrolysis of enkephalins (Hayashi, 1978) and endorphins (Hersh and McKelvy, 1981) or the degradation of angiotensin II (Kelly *et al.*, 1983), substance P (Benuck and Marks, 1975) and TRH (Torres *et al.*, 1986), may be performed by aminopeptidases. In the adult brain, these neuropeptides serve as neurotransmitters and/or neuromodulators. However, it has also been proposed that during embryonic development and the first stages postbirth, the NP could act as a growth regulatory factor, due to the fact that the concentrations of several kinds of this neurosecretory material are high at the embryonic stage and decline progressively in adulthood (Bartolomé *et al.*, 1986; Hayashi, 1987). The results obtained in this research show how both Leu- and Lys-AP activities increase from birth to the 1st week of life (except for the significant decline observed on the 3rd day). The increase of these enzymes possibly reflects decreases in the activity of neuropeptides, which coincides with previous reports, and moreover the changes are produced during axonal and dendritic growth. Nevertheless, it cannot be ruled out that the decrease in neuropeptide activity might also be due to the down-regulation of transcription or translation (Hayashi *et al.*, 1990).

During this developmental period, decreases in the AP activity have also been detected. The decline in enzyme activities was observed to occur between the 1st and the 3rd day of life. It is of interest that several parallel neurochemical changes occur at the same time (Leon *et al.*, 1982; Matsuo *et al.*, 1987). In any case, the changes in AP activities observed during axonal and dendritic growth are not so marked as those detected during myelination and the slow deposition of solids.

Of interest is the behaviour observed in the enzyme activities of the cerebellum and medulla during the 5th and 7th days postbirth. In these regions, there are no differences in the activities between these two stages, but in both cerebral hemispheres, there is a marked rise from the 5th to the 7th day. It has recently been suggested that, in the cerebellum, the concentration of some mRNA is differentially regulated with respect to other structures of the central nervous system (Hayashi *et al.*, 1990).

Finally, in all of the developmental stages under study, interhemispheric differences in the aminopeptidase activities were observed. Thus, the asymmetrical distribution of these enzymes seems to be limited to the cortex in the adult rat brain (Alba *et al.*, 1986).

Experimental Procedures

Male Sprague-Dawley rats, at 1, 3, 5 and 7 days of postnatal (n=10 each) 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.

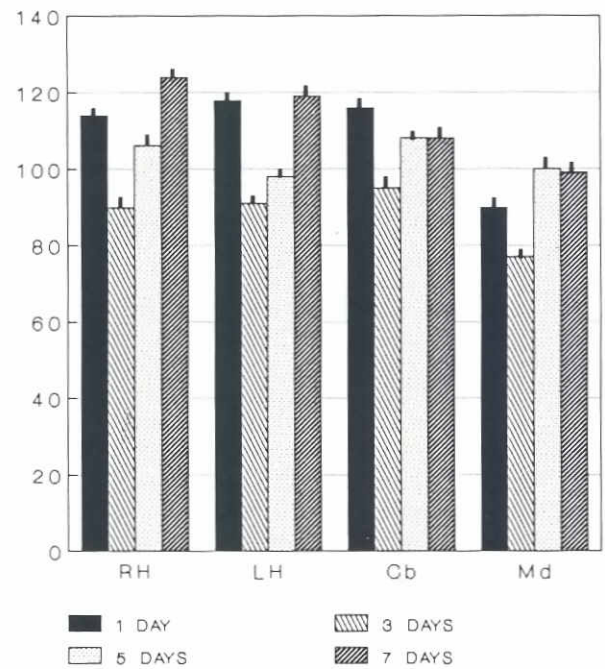

Fig. 2. Age-related changes in Leu-aminopeptidase activity in the right (RH) and left (LH) hemispheres, cerebellum (Cb) and medulla (Md). *Aminopeptidase activity was fluorimetrically measured in triplicate using Leu-2-naphthylamide as substrate. The results are expressed as units of aminopeptidase/mg protein. Each column is the mean for 10 animals, with the standard error.*

Brain samples were rapidly removed from the cerebral hemispheres, cerebellum and medulla. Samples were homogenized in 10 vol. of 10 mmol/l 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-2-naphthylamides (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.*, 1989): 10 µl aliquots of soluble fraction were incubated for 30 min with 1 ml of either Leu- or Lys-NA (1mg/dl), bovine serum albumin (10 mg/dl) and Dithiothreitol (10 mg/dl) in 50 mmol/l phosphate buffer, pH 7.4. The reaction was stopped by the addition of 1 ml of 0.1 mol/l 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 aminopeptidase per mg protein (mean±SEM). One unit of AP was defined as the amount of enzyme that hydrolyzes 1 pmol of Leu- 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 PLSD-Fisher's test. Comparison between groups was made using analysis of variance (ANOVA).

Acknowledgments

We would like to thank Prof. David Hallett for his revision of the manuscript.

References

ALBA, F., IRIBAR, C., RAMIREZ, M. and ARENAS C. (1989). A fluorimetric method for the determination of brain aminopeptidases. *Arch. Neurobiol. 52:* 169-173.

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.

BARTOLOME, J.V., BARTOLOME, M.B., DALTNER, L.A., EVANS, C.J., BARCHAS, J.D., KUHN, C.M. and SCHANBERG, S.M. (1986). Effect of B-endorphin 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.

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

CAMARGO, A.C.M., RAMACHO-PINTO, F.J. and GREENE, L.J. (1972). Brain peptidases: conversion and inactivation of kinin hormones. *J. Neurochem. 19:* 37-39.

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., MUGICA, R., ARECHAGA, G. and RAMIREZ, M. (1989a). Aging and brain aminopeptidase activity. *Rev. Esp. Fisiol. 45:* 89-94.

GANDARIAS, J.M. DE, IRAZUSTA, J., ECHEVARRIA, E., MUGICA, J. and CASIS, L. (1989b). Pre- and postnatal aminopeptidase activities in the rat brain. *Int: J. Dev. Biol. 33:* 491-493.

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

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. *Dev. Brain Res. 32:* 181-186.

HAYASHI, M., YAMASHITA, A., SHIMIZU, K., SOGAWA, K. and FUJII, Y. (1990). Somatostatin gene expression in the developing monkey frontal and cerebellar cortices. *Dev. Brain Res. 57:* 37-41.

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

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

LEON, M., BROQUET, P., MORELIS, R. and LOUISOT, P. (1982). Postnatal developmental changes in fucosyltranferase activity in rat brain. *J. Neurochem. 39:* 1770-1773.

MATSUO, K., MOSS, L. and HOMMES, F. (1987). Development of adenosine 5'-triphosphate sulfurylase and adenosine phosphosulfate kinase in rat cerebrum and liver. *Dev. Neurosci. 9:* 128-132.

TORRES, H., CHARLI, J., GONZALES-NORIEGA, A., VARGAS, M.A. and JOSEPH-BRAVO, P. (1986). Subcellular distribution of the enzymes degrading thyrotropin releasing hormone and metabolites in rat brain. *Neurochem. Int. 9:* 103-110.

TURZYNSKI, A. and MENTLEIN, R. (1990). Prolyl aminopeptidase from rat brain and kidney. *Eur. J. Biochem. 90:* 509-515.

Accepted for publication: June 1992