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Developmental and ageing changes in aminopeptidase activities in selected tissues of the rat

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Abstract. Aminopeptidase activities, assayed as arylamidase activities, were investigated in selected tissues of 1, 6, 12 and 24-month-old rats. The enzyme activities were found to have a heterogeneous distribution and age-related changes were observed. The highest levels of soluble arginyl-aminopeptidase activity were detected in brain homogenate at all the studied ages, whereas membrane-bound activity presented the highest levels in brain and kidney in the four ages tested. Aspartyl-aminopeptidase activity was detected mainly in the particulate fraction of kidney at all four ages. In 1, 6 and 12-month-old animals, soluble aspartyl-aminopeptidase activity was also higher in the kidney than in the rest of the tissues, whereas in the group of 2-year-old rats, the highest levels were found in both kidney and liver. Age-related changes were observed in all the studied tissues and for all the assayed enzymatic activities. In general, the maximal levels were detected in both the youngest and the oldest animals, and the minimal ones in 6 and 12-month-old rats. However, in the adrenals, the soluble and membrane-bound arginyl-aminopeptidase activity was higher in 6-month and 2-year-old rats than in 1-month and 12-month-old rats. These changes may reflect the functional status of the susceptible endogenous substrates of aminopeptidases.
Key words. Aspartyl aminopeptidase; arginyl aminopeptidase; β-naphthylamide; development; ageing; rat tissues.

Peptidases play a major role in the metabolism of several active peptides. Aspartyl-aminopeptidase (AspAP) (EC 3.4.11.-) removes the amino terminal Asp[1] from its substrates angiotensin (ANG) I and ANG II and produces des-Asp[1]-ANG I and ANG III, respectively[2]. The latter is then metabolized in part by arginyl-aminopeptidase (ArgAP) (EC 3.4.11.6)[3]. Both enzymes have been demonstrated to take part in the regulation of the physiological effects of ANG peptides[4,5]. It has been claimed that ArgAP plays a role in the metabolism of several active peptides such as Met-enkephalin[6,7], somatostatin, neurotensin, bradikinin, substance P and luliberin[8]. Furthermore, AspAP could also be involved in the metabolism of peptides other than ANG II which possess N-terminal Asp residues.

However, the actual physiological role of these enzymes is not well defined. The determination of the tissue distribution of these peptidase activities, and any changes that occur during development and ageing, could contribute to clarifying the regulatory mechanisms which control the activity of peptides susceptible to hydrolysis by these enzymes. The present study was conducted to quantify comparatively the levels of soluble and membrane-bound aspartyl- and arginyl-aminopeptidase activities in selected tissues, and to analyze age-related changes in their activities in 1, 6, 12 and 24-month-old rats. We used a-Asp-β-naphthylamide (AspNNap) and Arg-β-naphthylamide (ArgNNap) as substrates for the detection of aspartyl- and arginyl-aminopeptidase, respectively[9].

Materials and methods

1, 6, 12 and 24 months of age, Sprague-Dawley rats, were used in this study. The rats were housed at a constant temperature of 25 °C with lights on from 07.00 to 19.00 h and were given free access to laboratory chow and water. In order to avoid possible diurnal variations[10], all the experiments were performed at the same time of day (09.00 h). The animals were perfused with saline through the left cardiac ventricle, under equithensin anesthesia (2 ml/kg b.wt). Their brains, adrenals, and samples of lung, kidney and liver were quickly removed and immediately processed. Blood samples were obtained before perfusion from the left cardiac ventricle. Tissue samples from the same animal were always processed in the same assay, and all the enzymatic activities were determined in each tissue sample.

Total brain, adrenals, and tissue samples were homogenized separately, without pooling, in 10 volumes of Tris-HCl 10 mM (pH 7.4), and centrifuged (100,000 × g, 30 min, 4 °C). Samples from these supernatants were used to determine soluble activity and proteins, assayed in triplicate. The resultant pellets were homogenized in Tris-HCl 10 mM (pH 7.4) plus 1% of Triton-X-100 to obtain, after centrifugation (100,000 × g, 30 min, 4 °C), supernatants which were employed to determine membrane-bound activity and proteins, also in triplicate.

Arginyl-aminopeptidase activity was measured in a fluorometric assay[11], employing ArgNNap (Sigma

Chemical, St. Louis, MO) as substrate, as previously described[11]. The assay was modified as follows: 10 µl of supernatant was incubated for 30 min at 25 °C with 1 ml of the substrate solution [1 mg/100 ml ArgNNap, 10 mg/100 ml bovine serum albumin and 10 mg/100 ml dithiothreitol (Sigma Chemical, St. Louis, MO) in 10 mM phosphate buffer pH 7.4]. Aspartyl-aminopeptidase activity was measured in a fluorometric assay[12], employing AspNNap (Sigma Chemical, St. Louis, MO) as substrate, modified as follows: 10 µl portions of supernatants were incubated for 120 min at 37 °C with 1 ml of the substrate solution (1 mg/100 ml AspNNap, 10 mg/100 ml bovine serum albumin and 10 mg/100 ml MnCl2 in Tris-HCl 50 mM, pH 7.4). After the incubations, all the enzymatic reactions were stopped by adding 1 ml 0.1 M acetate buffer (pH 4.2). The quantity of β-naphthylamine released as a result of the enzymatic activity was determined fluorometrically at 412 nm of emission wavelength with an excitation wavelength of 345 nm. The protein concentration was measured by a protein-dye binding assay, colorimetrically determined by absorbance at 595 nm (Bio-Rad Laboratories, Richmond, CA)[13]. Specific arginyl- and aspartyl-aminopeptidase activities were expressed respectively as nmol of ArgNNap and pmol of AspNNap hydrolyzed per min per mg of protein. Fluorogenic assays were linear with respect to time of hydrolysis and protein content.

Results are presented as means ±SEM. Comparisons between means were made using the unpaired Student's t test and values of $p < 0.05$ were considered significant.

Results

Values of soluble (Sol) and membrane-bound (M-B) arginyl- and aspartyl-aminopeptidase activities, in different tissues of 1, 6, 12 and 24-month-old rats, are presented in table 1. The statistical differences between means are indicated in table 2. Wide differences among tissues in all the analyzed enzymatic activities were found. The highest levels of Sol arginyl-aminopeptidase activity were detected in the brain in all animals, regardless of their age ($p < 0.001$). The M-B activity of arginyl-aminopeptidase was also predominant in brain homogenate of 1-month-old rats ($p < 0.025$ when compared with kidney and $1 < 0.001$ when compared with the rest of the tissues). In 6-month-old rats the highest levels of this enzymatic activity were observed in brain (brain vs rest $p < 0.001$). In 12-month-old ($p < 0.01$) and 2-year-old rats ($p < 0.001$) the highest levels were in brain and kidney, without differences between the two organs.

The soluble form of aspartyl-aminopeptidase activity was higher in the kidney than in the rest of the tissues in 1-month-old rats ($p < 0.001$). In 6-month-old rats the highest levels were found in kidney ($p < 0.001$). In 12-month-old rats the highest levels also corresponded to kidney ($p < 0.025$ vs liver and $p < 0.001$ vs rest). In aged rats, the highest levels were observed in kidney and liver ($p < 0.001$ when comparing kidney with the rest, $p < 0.025$ when comparing liver with lung, and $p < 0.001$ when comparing liver with the rest of the tissues). The M-B aspartyl-aminopeptidase activity was predominantly detected in the kidney in the four ages tested ($p < 0.001$).

Statistical comparisons between ages showed significant differences in all the tissues and enzymatic activities assayed. In all the tissues and organs studied, with the exception of the adrenal glands, the maximal levels of activity were observed in young and aged animals and the minimal ones in 6 and 12-month-old rats. In adrenals, the Sol and M-B arginyl-aminopeptidase activity was higher in 6-month and 2-year-old rats than in 1- and 12-month-old rats.

Discussion

The analysis of the pattern of distribution of aspartyl- and arginyl-aminopeptidase activities and the study of their putative changes during development and ageing can provide useful information for a deeper understanding of the physiological roles of these enzymes and their substrates. The results of the present study demonstrate a heterogeneous distribution of aspartyl- and arginyl-aminopeptidase activities in several tissues of the rat, which implies distinct biological activities of these enzymes in different tissues and organs. Since AspAP has been suggested to play a physiological role as an angiotensinase[12], the high levels of membrane-bound activity in the kidneys, described for the first time in the present study, strongly suggest a main role for this tissue in the inactivation of circulating ANG II. ArgAP exhibited a different pattern of tissue distribution, which indicates that there is not a coordinated action of this enzyme with AspAP in ANG metabolism. Also, because of its broad substrate specificity, there is not a clear relationship between ArgAP activity and the metabolism of any of its putative substrates.

With respect to the developmental modification of these enzymatic activities, the results presented here demonstrate significant age-related changes of ArgAP and AspAP in several organs of developing rats. Except in the adrenals, peptidase activities were found to be higher in both young (1-month-old) and aged (24-month-old) rats than in adult animals (6 and 12 months old), although the mechanisms which underlie this pattern remain unclear. Peptidase activities could reflect substantial hormonal and metabolic changes occurring in these contrasting periods of life. As can be noticed in table 1, in most tissues, aminopeptidase activities decrease in 6-month-old rats, reaching a minimum in 12-month-old animals, once the plateau of adult homeostasis has been fully reached. However, in 1-month-old animals – that is, in early development when they are not yet sexually mature – as well as in aged rats, when sexual activity declines,

Table 1. Aminopeptidase activities in various tissues of 1, 6, 12 and 24-month-old male Sprague-Dawley rats

Enzymatic activity	Age	Lung	Kidney	Liver	Adrenal	Brain	Serum
Sol ArgAP	1 m	14.5 ± 1.2 (11)	25.9 ± 1.2 (11)	12.5 ± 0.8 (11)	13.3 ± 0.28 (12)	40.8 ± 5.3 (6)	0.25 ± 0.01 (8)
	6 m	16.6 ± 2 (5)	20.1 ± 1.7 (5)	11.4 ± 1 (5)	19.1 ± 0.59 (10)	37.5 ± 0.4 (5)	0.2 ± 0.01 (5)
	12 m	7.9 ± 1 (5)	15.2 ± 1.2 (5)	8.8 ± 1.1 (5)	10.6 ± 0.3 (10)	30.1 ± 0.4 (5)	0.066 ± 0.007 (5)
	24 m	12.9 ± 0.8 (11)	20 ± 1.7 (11)	13.6 ± 1.1 (10)	15.8 ± 0.7 (11)	37.5 ± 2.9 (14)	0.14 ± 0.02 (9)
M-B ArgAP	1 m	3.7 ± 0.4 (11)	17.6 ± 0.9 (11)	3.3 ± 0.3 (11)	1.7 ± 0.13 (12)	23.3 ± 2.2 (8)	
	6 m	5.7 ± 1.1 (5)	11.5 ± 0.9 (5)	2.1 ± 0.4 (5)	2.4 ± 0.1 (10)	19.3 ± 1.4 (5)	
	12 m	2.6 ± 0.3 (5)	13.1 ± 0.25 (5)	1.9 ± 0.22 (5)	1.3 ± 0.1 (10)	12.4 ± 1.8 (5)	
	24 m	3.9 ± 0.3 (11)	14.3 ± 1 (9)	2.9 ± 0.5 (9)	2.3 ± 0.2 (11)	16.8 ± 1.9 (13)	
Sol AspAP	1 m	121 ± 25 (11)	420 ± 81 (11)	125 ± 20 (11)	60.8 ± 6.3 (12)	14.4 ± 2 (6)	7.3 ± 0.8 (8)
	6 m	47 ± 2.7 (5)	119 ± 9 (5)	63.8 ± 8.8 (5)	39.7 ± 4.3 (10)	23.7 ± 8.8 (5)	4.7 ± 2 (5)
	12 m	38.4 ± 8.8 (5)	120 ± 14 (5)	65.2 ± 5.5 (5)	29.9 ± 3.7 (10)	10.7 ± 1.1 (5)	2.4 ± 0.4 (5)
	24 m	104 ± 10 (11)	269 ± 40 (10)	238 ± 48 (11)	64.9 ± 6.7 (11)	25.6 ± 3.4 (12)	7 ± 1.2 (9)
M-B AspAP	1 m	423 ± 108 (8)	2,840 ± 560 (11)	295 ± 75 (11)	128 ± 15 (10)	39.5 ± 6.9 (6)	
	6 m	80.9 ± 20.7 (5)	589 ± 106 (5)	53.1 ± 9.5 (5)	54.7 ± 5.1 (10)	33.8 ± 1.1 (5)	
	12 m	53 ± 12.3 (5)	617 ± 100 (5)	45.1 ± 4.2 (5)	46.6 ± 3.2 (10)	30.1 ± 1.4 (5)	
	24 m	322 ± 55 (10)	2,385 ± 476 (7)	206 ± 52 (9)	145 ± 12 (11)	51.2 ± 5.4 (12)	

Values represent mean ± SEM for groups of 5 to 14 animals assayed individually. Number of determinations in brackets. Sol, soluble; M-B, membrane-bound; ArgAP, arginyl-aminopeptidase (nmol/min/mg prot); AspAP, aspartyl-aminopeptidase (pmol/min/mg prot); 1 m, 1-month-old; 6 m, 6-month-old; 12 m, 12-month-old; 24 m, 24-month-old.

Table 2. Statistical differences of aminopeptidase activities between ages in each localization

Enzymatic activity	Age	Lung			Kidney			Liver			Adrenal			Brain			Serum		
		6 m	12 m	24 m	6 m	12 m	24 m	6 m	12 m	24 m	6 m	12 m	24 m	6 m	12 m	24 m	6 m	12 m	24 m
Sol ArgAP	1 m	NS	**	NS	*	***	*	NS	*	NS	***	***	***	NS	NS	NS	**	***	***
	6 m		**	NS		NS	NS		NS	NS		***	***		***	NS		***	NS
	12 m			***			NS			**			***			NS			*
M-B ArgAP	1 m	NS	NS	NS	***	**	*	*	*	NS	***	*	*	NS	**	NS			
	6 m		*	NS		NS	NS		NS	NS		***	NS		*	NS			
	12 m			*			NS			NS			***			NS			
Sol AspAP	1 m	*	*	NS	*	*	NS	NS	NS	NS	*	***	NS	NS	NS	NS	NS	***	NS
	6 m		NS	***		NS	*		NS	*		NS	**		NS	NS		NS	NS
	12 m			***			*			*			***			*			*
M-B AspAP	1 m	*	*	NS	*	*	NS	*	*	NS	***	***	NS	NS	NS	NS			
	6 m		NS	*		NS	*		NS	*		NS	***		NS	NS			
	12 m			**			*			*			***			*			

Comparisons between means were made using the unpaired Student's t test: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; NS, not significant. See table 1 for abbreviations.

aminopeptidase activities reach a peak. The developmental pattern is opposite to those of testosterone synthesis and release, which increase in the early stages of development and diminish in old age[14], which suggests that sexual steroids may exert some influence on aminopeptidase activity and, in consequence, on the functional status of their substrates. In support of this hypothesis, previous studies have demonstrated an increase in aminopeptidase activity after orchiectomy in male rats[15].

The developing profile of Sol and M-B arginyl-aminopeptidase activities in adrenals differs from the patterns shown by the rest of the tissues as well as from that of other activities tested. This fact implies a differential regulatory mechanism of their substrates in this localization, although its functional meaning is, at present, unknown.

Further determinations of the specific localizations of these activities within each organ are required for a better understanding of their functional role.

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