

SHORT COMMUNICATIONS

A Bilateral Study of Aminopeptidase Activity in the Adrenal Gland of Young and Aged Rats

A local renin-angiotensin system (RAS) with high levels of angiotensin II (ANG II) has been described in the adrenal gland. However, its physiological role is not yet understood (6). In the ANG metabolism several peptidases have been involved including aspartyl aminopeptidase (AspAP) (4) and arginyl aminopeptidase (ArgAP) (1). Previous studies on the regulation of the RAS in the adrenal gland have paid little attention to the angiotensinase activity of AspAP and ArgAP. In order to investigate the effect of age on these activities, the levels of soluble (S-) and membrane-bound (MB-) AspAP and ArgAP activities were determined in the left and right adrenals of young (one-month-old) and aged (two-year-old) male rats, using Asp- β -naphthylamide (AspN-Nap) and Arg- β -naphthylamide (ArgN-Nap) as substrates. For purposes of comparison, the corticosterone content was determined in parallel from the soluble fractions.

Rats were anaesthetized with equithen-sin and perfused with saline through the left cardiac ventricle. The left and right adrenals were quickly removed and homogenized separately in 500 μ l of Tris-HCl 10 mM (pH 7.4). The homogenates were then centrifuged (100,000 g, 30 min, 4 °C) and samples from the supernatants were used for determinations of soluble enzymatic activity, protein and corticosterone content. The pellets were homogenized in 500 μ l of Tris-HCl 10 mM (pH 7.4) plus 1 % Triton-X-100 to obtain, after centrifugation (100,000 g, 30 min, 4 °C), supernatants which were used to

determine membrane-bound activity and proteins. ArgAP and AspAP activities were measured with a fluorometric assay as previously was described (7, 8). Proteins were quantified by the method of BRADFORD (3). ArgAP and AspAP activities were expressed respectively as nmol of ArgN-Nap and pmol of AspN-Nap hydrolysed per min per mg of protein. Corticosterone content was determined by radioimmunoassay as previously reported (2), and expressed as ng per mg of protein (table I). For left-right comparisons, the Wilcoxon signed rank test was used. All p values are two-tailed. For age-comparisons, the Wilcoxon rank sum test was applied. Values of $p < 0.05$ were considered significant.

Table I. Specific soluble and membrane-bound aminopeptidase activities and corticosterone content in the left (L) and right (R) adrenals of one-month old (1M) and two-year old (2Y) rats.

Values represent mean \pm SEM of groups of 7-12 animals assayed individually.

		1M	2Y
S-AspAP	L	67 \pm 9.2	55 \pm 6.6
	R	54.5 \pm 9.0	47.4 \pm 6.5
MB-AspAP	L	133 \pm 23	174 \pm 27
	R	123 \pm 24	155 \pm 20
S-ArgAP	L	13.7 \pm 0.3	15.7 \pm 0.9
	R	13 \pm 0.4	15.7 \pm 1.1
MB-ArgAP	L	1.8 \pm 0.2	2.7 \pm 0.5
	R	1.6 \pm 0.1	2.9 \pm 0.5
Corticosterone	L	128 \pm 18	182 \pm 47
	R	195 \pm 24	247 \pm 34

In one-month-old rats, specific S-AspAP activity in the left adrenal was significantly higher than in the right ($p = 0.0035$), whereas corticosterone content was significantly higher in the right gland than the left one ($p = 0.039$). In two year-old rats, no differences were observed for any of the parameters between left and right adrenals. Comparisons between the two ages showed an increase of SArgAP activity in the left ($p = 0.03$) and right ($p = 0.009$) adrenals of two-year-old animals, and an increase of MB-ArgAP activity only in the right ($p = 0.004$) adrenal of two-year-old rats. The activity of MB AspAP was higher than that of the soluble fraction in both age groups. However, S-ArgAP exhibited higher activity than the membrane-bound fraction in both ages.

Because whole glands were used, with no distinction between cortical and medullary activities, the data reported herein may reflect both cortical and adreno-medullary processing of susceptible substrates. Except for the well known predominance of solitary pheochromocytomas in right adrenal gland (5), no other anatomical or biochemical asymmetries have been described in adrenals as yet. We report significant left-right differences in S-AspAP activity and corticosterone content in one-month-old rats. However, in older animals, no right vs left differences were observed in any of the parameters studied. These findings raise the possibility of asymmetrical functioning of the endogenous substrates of S-AspAP in young adrenal glands. Since specific asymmetries were found in young animals (S-AspAP and corticosterone), with opposite left-right distribution, a general acceptable explanation, such as left-right differences in weight or flow rate, cannot be outlined and they support more selective functional differences between the two glands. In addition, significant age-related changes occur in the S- and MB-forms of ArgAP activity which could reflect a modification in their action over susceptible substrates.

Key words: Asymmetry, Aging, Adrenal, Amino-peptidase, Corticosterone.

Palabras clave: Asimetría, Envejecimiento, Adrenal, Amino-peptidasa, Corticosterona.

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(Received on January 10, 1994)

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