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Dipeptidyl aminopeptidase in neonatal rat brain regions

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Summary. Changes in the activity of dipeptidyl aminopeptidase in rat pituitary gland and various brain regions were examined at 3, 5 and 20 weeks of age. The enzyme activity per g tissue wet weight in pituitary gland was the highest of all tissues studied. Subcellular distribution of the activity was also studied. The highest enzyme activity was found in a crude mitochondrial fraction including synaptosomes.

Dipeptidyl aminopeptidase belongs to the group of intracellular enzymes that are capable of cleaving dipeptide moieties from the unsubstituted NH_2 termini of peptides and dipeptidyl arylamides. Developmental changes in dipeptidyl peptidase I and III activities during maturation of rat brain have been studied by Marks et al.[1]. We have also found dipeptidyl aminopeptidase activity in developing rat brain by using 7-(Gly-Pro)-4-methylcoumarinamide (Gly-Pro-MCA) as substrate[2]. Although dipeptidyl aminopeptidase II has an optimum pH between 5.0 and 6.0[3], similar to that of rat brain dipeptidyl aminopeptidase, properties of both enzymes are slightly different from each other[2]. In a previous report[2], we found that the level of dipeptidyl aminopeptidase in the young whole rat brain was higher than that in the adult rat brain. However, the real physiological role of this enzyme is little known. In this paper, we describe regional and subcellular distributions of the enzyme in developing rat brain in order to ascertain its physiological role.

Materials and methods. Timed pregnant Sprague-Dawley rats were obtained from Charles River Laboratories in Japan. Each animal was killed by decapitation. Brain tissues were quickly separated and homogenized in 9 volumes of 0.32 M sucrose. Subcellular fractions were prepared according to the method of Gray and Whittaker[4]. Enzyme activity was assayed with Gly-Pro-MCA as reported previously[5], but using Britton-Robinson's universal buffer, pH 6.0. Incubation mixture (total volume 100 µl) contained 20 µl of 1 mM Gly-Pro-MCA, 20 µl of Britton-Robinson's universal buffer (pH 6.0) and enzyme. Incubation was performed at 37 °C for 5 min and the reaction was stopped by adding 1 ml of 1 M acetate buffer, pH 4.2. 7-amino-4-methylcoumarin (AMC) liberated from Gly-Pro-MCA was determined by the fluorescence intensity at 460 nm with excitation at 380 nm.

1 unit (U) of enzyme was defined as the amount of enzyme which released 1 µmol of AMC per min from the Gly-Pro-MCA.

Results and discussion. As shown in table 1, the activity of dipeptidyl aminopeptidase in pituitary gland was 2–5 times higher than that in the other brain regions studied. The

Table 1. Regional distribution of dipeptidyl aminopeptidase activity in rat brain and pituitary gland

Tissue	Enzyme activity (U/g tissue)		
	3 weeks	5 weeks	20 weeks
Cerebellum	0.585 ± 0.062	0.560 ± 0.039	0.272 ± 0.036
Cerebrum	0.405 ± 0.103	0.344 ± 0.029	0.251 ± 0.058
Colliculi	0.421 ± 0.081	0.407 ± 0.031	0.282 ± 0.054
Pons medulla	0.611 ± 0.171	0.468 ± 0.018	0.263 ± 0.019
Midbrain	0.488 ± 0.111	0.391 ± 0.049	0.231 ± 0.007
Hypothalamus	0.415 ± 0.104	0.326 ± 0.078	0.276 ± 0.024
Pituitary gland	1.084 ± 0.027	1.333 ± 0.098	1.234 ± 0.115

Each value represents mean ± SEM, n = 3.

enzyme activity in pituitary gland at 5 weeks of age was the highest among the three ages tested. However, in the other brain regions, the activity of this enzyme was highest at 3 weeks old. These results confirm previous data concerning the developmental changes in the activity of rat whole brain[2]. These changes in developing rat whole brain are unique for the dipeptidyl aminopeptidase represented here, while other dipeptidyl aminipeptidases (dipeptidyl peptidase I and III) showed little changes in developing rat whole brain[1]. In various rat brain regions, the activity in the pons-medulla was slightly higher at 3 weeks old, but was similar to other brain regions at 5 and 20 weeks (table 1).

Changes in the enzymatic activity in developing rat brain regions were similar to those in the whole brain. These results indicate that dipeptidyl aminopeptidase has a broad distribution, but do not show whether the enzyme is located in neuronal or in glial cells.

Table 2. Subcellular distribution of dipeptidyl aminopeptidase activity in rat cerebrum, hypothalamus, midbrain and pons medulla

Subcellular fraction		Percent of total activity			
		1 week	3 weeks	5 weeks	20 weeks
Cerebrum	P_1	(17.7)	20.0	9.0	23.1
	P_2	(54.0)	36.8	44.5	33.9
	P_3	(6.6)	6.2	6.1	6.0
	Sup	(21.7)	15.8	8.1	11.6
Hypothalamus	P_1	(22.3)	18.3	12.9	17.8
	P_2	(61.5)	30.1	23.0	33.7
	P_3	(3.5)	0.7	1.8	1.1
	Sup	(12.7)	22.7	21.5	18.8
Midbrain	P_1	(18.3)	21.3	9.2	26.8
	P_2	(48.9)	29.3	42.2	30.7
	P_3	(8.8)	6.8	9.7	6.1
	Sup	(24.0)	11.9	10.7	15.6
Pons medulla	P_1	(24.0)	18.2	9.0	24.3
	P_2	(51.0)	26.7	39.1	35.0
	P_3	(7.2)	3.9	8.8	4.2
	Sup	(17.8)	11.5	11.3	10.6

Values were calculated from total activity in a homogenate fraction, and represent means from 3 experiments.
The values for 1-week-old rat brain, given in parentheses, were estimated as a percentage of the gross activity in each fraction.
Symbols used in this table represent subcellular fractions; P_1, P_2 and P_3, pellets at 700 × g, 10,000 × g, and 100,000 × g, respectively; Sup, supernatant at 100,000 × g.

Subcellular distribution of dipeptidyl aminopeptidase in 4 brain regions is shown in table 2. The activity in the P_1 (nuclear) fraction is slightly high, but this might result from contamination with intact cells in addition to nulei and cell debris. The activity in the P_2 (mitochondrial) fraction is about one half of the total activity in each of the brain regions studied, and the activity in the P_3 (microsomal) fraction is the lowest in the 4 fractions, during maturation of rat brain. The P_2 fraction consists of synaptic vesicles and myelin, in addition to membrane debris, mitochondria and lysosomes, so it is probable that dipeptidyl aminopeptidase in the brain is present in the nerve endings. In this study, rather high enzyme activity is observed in the supernatant fraction. It may be derived from the activity in particles of the P_2 fraction because of the similarity in optimum pH (data not shown). Since rat brain dipeptidyl aminopeptidase attacks peptide bonds between *N*-terminal X-Pro dipeptides (X: amino acid residues) and amino acid or arylamide, a neuropeptide containing the X-Pro sequence at the *N*-terminal position may be susceptible to hydrolysis by this enzyme. These results indicate the possibility that the processing of biologically active peptides occurs in the nervous system.

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A comparison between skin-photosensitizing (334 nm) activities of 8-methoxypsoralen and angelicin

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Summary. 8-Methoxypsoralen and angelicin, locally applied to the skin of the rabbit, photosensitized erythema under UV irradiation to an approximately equal extent. Incident UV irradiation was restricted mainly to 334 nm. This indicates that furocoumarin-DNA cross-links are not important in erythema induction.

Furocoumarins (psoralens) photosensitize erythema of the mammalian skin under near-UV irradiation (UVA, 315–400 nm)[2]. The linear furocoumarins such as 8-methoxypsoralen (8-MOP) are known to photosensitize erythema under monochromatic light (366 nm) much better than those with the angular structure, for instance, angelicin[2,3]. This difference was thought to be due to the ability of 8-MOP to form cross-links with DNA under UVA-irradiation, whereas angelicin has no such ability[4,5]. It should, however, be noted that molar absorbtion coefficients (ε_{366}) for 8-MOP and angelicin at 366 nm are 1000 and 60 $1 \times mol^{-1} \times cm^{-1}$, respectively. This means that the lower photosensitizing activity of angelicin, compared to that of 8-MOP, may be due to its lower absorbance at 366 nm, and not due to the inability of angelicin to form cross-links.

The purpose of the present paper is to compare the photosensitizing activities of 8-MOP and angelicin irradiated at 334 nm, where the ε_{334} values of both are approximately equal at 4000 $1 \times mol^{-1} \times cm^{-1}$.

8-MOP and angelicin were kindly supplied by Professor G. Rodighiero (Padova, Italy).

Albino rabbit skin was used. The rabbit's back was shaved