Effects of thyroidectomy on monoamine oxidase activities toward tyramine and serotonin in the circumventricular nuclei of the rat

J. S. Kim, M. Hirano, H. Uchimura, M. Saito, T. Nakahara and M. Itoh

Laboratory of Neurochemistry, HIZEN National Mental Hospital, Kanzaki, Saga, 842-01 (Japan), Department of Chemistry, Faculty of Science, Kyushu University, Hakozaki, Fukuoka, 812 (Japan), and Department of Neuropsychiatry, Faculty of Medicine, Kyushu University, Katakasu, Fukuoka, 812 (Japan), 11 July 1978

Summary. Following thyroidectomy, monoamine oxidase (MAO) activities toward tyramine decreased significantly by 20% in the nucleus periventricularis and the nucleus arcuatus among the 3 hypothalamic nuclei of the rat, while MAO activity toward serotonin decreased significantly by 10% only in the nucleus periventricularis. It is suggested that thyroidectomy induced selective changes on the multiple forms of MAO in the discrete circumventricular nuclei.

Monoamine oxidase (MAO) (E.C. 1.4.3.4.), which is important for the metabolism of various brain monoamines, has been found to occur in multiple forms, and designated as type A MAO and type B MAO based on substrate specificity, inhibitor sensitivity and thermal stability[1,2]. Type A MAO deaminates preferentially norepinephrine and serotonin, while type B MAO deaminates preferentially β-phenylethylamine and benzylamine. Tyramine is deaminated by both types of MAO.

We found, in recent years, extremely high concentrations of MAO toward tyramine (Tyr-MAO) and strikingly low values of MAO toward serotonin (5HT-MAO) to Tyr-MAO ratios in the discrete circumventricular regions of the rat, and suggested that the circumventricular regions were generally rich in type B MAO[3,4].

Morphological[5], immunohistochemical[6] and physiological[7,8] evidence suggests that the ependymal cells on the surface of the ventricle are involved in endocrine regulation by participating in the transport function of various biogenic amines and some hypothalamic hormones in the cerebrospinal fluid (CSF) to pituitary portal capillaries. It is of interest, therefore, to study the physiological roles of type B MAO in the circumventricular regions. This study describes the effects of thyroidectomy on Tyr-MAO and 5HT-MAO in the circumventricular nuclei of rat medial hypothalamus, using the microdissection technique with freeze-dried sections[9] and radiomicromethods[3,4,10].

Materials and methods. Male Wistar-King rats (3 months old) weighing 250–300 g, housed in a group, were used for the experiment. The animals were killed at 14.00 h. by decapitation 4 days after thyroidectomy or sham thyroidectomy under nembutal anesthesia. The part containing hypothalamus was isolated and frozen in liquid nitrogen. Frontal sections of 200 µm thickness were made in a cryostat at $-15\,^{\circ}C$. The sections were completely freeze-dried overnight at $-30\,^{\circ}C$ and 10^{-3} mm Hg and stored in tubes at $-20\,^{\circ}C$ under vacuum until use.

The 3 medial hypothalamic nuclei (the nucleus periventricularis, the nucleus arcuatus and the median eminence) were carefully dissected freehand under a stereomicroscope using the atlas of König and Klippel[11]. Each sample was weighed by an electronic microbalance (Type 4125, Sartorius). The sensitivity of this balance is 0.1 µg. The weight of each sample was 4–12 µg.

MAO activity was measured by a modification[3,4] of the radiochemical micromethod of McCaman et al.[10]. 3 µl of 0.05% Triton X-100 containing 0.05% BSA was added to the dry sample in a microtube. After the preincubation for 15 min at 0 °C, 2 µl of ice-cold buffer substrate was added to each tube (final concentrations; 0.1 M-potassium phosphate buffer, pH 7.95, 1.5 mM[^{14}C] tyramine hydrochloride, 9.2 mCi/mmole, New England Nuclear Co.). The experimental and blank (containing no enzyme) tubes were incubated at 38 °C for 30 min. The reaction was stopped by the addition of 1 µl of 3 N HCl. Radioactive metabolites were extracted by 50 µl of ethyl acetate. Radioactivity was determined in 10 ml of scintillator toluene solution by a liquid scintillation spectrometer. The counting efficiency was 72%. The same procedure was carried out with serotonin as substrate (final concentrations; 1.0 mM [2-^{14}C]5-hydroxytryptamine binoxalate, 48.5 mCi/mmole, New England Nuclear Co., 0.1 M potassium phosphate buffer, pH 7.2: incubation time, 15 min). Preliminary experiments indicated that the reaction with both substrates was linear for 30 min and up to 200 µg wet weight of whole brain tissue. Using this assay, the experimental values were at least 10 times the blank values (100 cpm).

Results and discussion. Our results are summarized in the table. Following thyroidectomy, Tyr-MAO activities decreased significantly by more than 20% in the nucleus periventricularis and the nucleus arcuatus. On the other hand, 5HT-MAO activity decreased significantly by 10% in the nucleus periventricularis but no significant changes were found in the nucleus arcuatus and the median eminence.

In the nucleus arcuatus, the decrease of Tyr-MAO following thyroidectomy reflects the change of type B MAO, since 5HT-MAO activity was not changed in the nucleus. On the other hand, in the nucleus periventricularis, the decrease of Tyr-MAO may reflect the changes of both types of MAO but mainly of type B MAO, since 5HT-MAO activity decreased slightly (10%) following thyroidectomy, and also it was found recently in our laboratory that only 30% of the total Tyr-MAO activity could be attributable to type A MAO in the nucleus periventricularis based on inhibition studies with clorgyline, a specific type A MAO inhibitor (unpublished data). Regarding the effects of hormones on the multiple forms of MAO, some workers have reported

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Nucleus of hypothalamus	Tyr-MAO (type A + B)		5HT-MAO (type A)	
	Sham-thyroidectomy	Thyroidectomy	Sham-thyroidectomy	Thyroidectomy
Nucleus periventricularis	234.34± 9.65 (4)	179.34± 9.35 (5)**	88.75±0.94 (4)	77.75±4.03 (5)*
Nucleus arcuatus	236.08± 1.81 (4)	183.84± 6.00 (5)**	74.76±2.53 (4)	69.51±1.47 (4)
Median eminence	145.75± 10.59 (4)	151.08± 10.91 (4)	59.58±1.50 (4)	61.69±2.21 (4)

Values (µmoles of product formed/g dry wt/h) represent the mean ± SEM with the numbers of animals in brackets. Double determinations were performed on each animal. Significance was determined by Student's t-test. * $p < 0.05$, ** $p < 0.005$.

recently that thyroid hormones acted discriminatively on different types of MAO in the rat heart[12], and that selective effects of oestradiol benzoate to ovariectomized rats on type A MAO were found in the basomedial hypothalamus and the corticomedial amygdala[13]. Similarly, present results suggest that thyroidectomy induced selective changes on the multiple forms of MAO in the discrete circumventricular nuclei. Further studies with a specific substrate for type B MAO, such as β-phenylethylamine, will be needed to ascertain the selective effects on type B MAO in the circumventricular nuclei, since tyramine is deaminated by both types of MAO.

It is known that all these nuclei are located close to the 3rd ventricle, and that the ependymal cells are distributed on the surface of these nuclei[5-7]. Furthermore, it is suggested that some hypothalamic hormones in the CSF are transported to hypophyseal portal blood mediated by the ependymal cells[6-8]. If it is assumed that the high amounts of type B MAO in these circumventricular nuclei are contained in the ependymal cell layer, the significant changes of type B MAO in the nucleus arcuatus and the nucleus periventricularis may be related to the changes of the transport function of the ependymal cells in these circumventricular nuclei.

1 J.P. Johnston, Biochem. Pharmac. *17*, 1285 (1968).
2 H.-Y.T. Yang and N.H. Neff, J. Pharmac. exp. Ther. *189*, 733 (1974).
4 M. Hirano, H. Uchimura and M. Saito, Brain Res. *93*, 558 (1975).
4 M. Hirano, J.S. Kim, M. Saito, H. Uchimura, M. Ito and T. Nakahara, J. Neurochem. *30*, 263 (1978).
5 J. Schechter and R. Weiner, Anat. Rec. *172*, 643 (1972).
6 E.A. Zimmerman, K.C. Hsu, M. Fersin and G.P. Kozlowski, Endocrinology *95*, 1 (1974).
7 H. Uemura, T. Asai, M. Nozaki and H. Kobayashi, Cell Tiss. Res. *160*, 443 (1975).
8 C. Oliver, N. Ben-Jonathan, R.S. Mical and J.C. Porter, Endocrinology *97*, 1138 (1975).
9 H. Uchimura, M. Hirano, M. Saito, A. Mukai and H. Hazama, Brain Nerve (Tokyo) *26*, 341 (1974).
10 R.E. McCaman, M.W. McCaman, J.M. Hunt and M.S. Smith, J. Neurochem. *12*, 15 (1965).
11 J.F.R. König and R.A. Klippel, The Rat Brain. Williams and Wilkins, Baltimore 1963.
12 J.H. Tong and A. D'Iorio, Endocrinology *98*, 761 (1976).
13 V.N. Luine and B.S. McEwen, J. Neurochem. *28*, 1221 (1977).

Tissue ascorbic acid and a liver dehydroascorbatase in guinea-pigs

Eleri Jones[1], J.E.W. Davies and R.E. Hughes

University of Wales Institute of Science and Technology, Cardiff CF1 3NU (Wales), 18 June 1978

Summary. The extraction, partial purification and assay of a dehydroascorbatase from guinea-pig liver is described. There was no evidence that changes in dehydroascorbatase activity could account for the modified tissue ascorbic acid concentrations associated with ageing or with the ingestion of fluoride, flavonoids or anthocyanin material.

Ascorbic acid (L-xyloascorbic acid, AA) is a labile component of most tissues and its concentration, in those species unable to synthesize it, is primarily a reflection of its intake in the diet. Tissue concentrations of AA may also, however, be substantially modified by a number of metabolic and physiological factors such as dietary composition, growth rate, metabolic activity, age, sex etc.[2,3]. The immediate determinants in the modification of tissue AA are largely uncharacterized but it would appear that a) the retention capacity of tissues and b) the rate of AA metabolism and breakdown are significant factors.

The catabolic pathways for tissue AA have not been completely elucidated and it would appear that considerable interspecies differences exist[4,5]. Ascorbic acid, at physiological conditions of pH and temperature, is rapidly converted to dehydroascorbic acid. Dehydroascorbic acid however is unlikely to attain any significant concentration in the tissues because of a) the ability of tissues to reduce it back to AA[6] and b) the presence of factors catalysing its further breakdown.

The enzyme glutathione: dehydroascorbate oxidoreductase (E.C. 1.8.5.1) which catalyzes the reduction of dehydroascorbic acid to AA in the presence of thiol compounds has been characterized in plants and there is evidence that a similar system exists in animal tissues[6]. A dehydroascorbatase catalyzing the delactonization of dehydroascorbic acid to diketogulonic acid, has been characterised in animal tissues[7]. The relative activities of these 2 systems could, in part, determine the concentration of AA in tissues; inhibition of dehydroascorbic acid breakdown would presumably result in elevated tissue AA concentrations and vice versa.

This note describes studies designed to determine whether nutritional and environmental factors shown to change AA concentrations in guinea-pig tissues were likely to operate by modifying a dehydroascorbatase system.

Table 1. Stability of dehydroascorbic acid (10^{-3} M) in phosphate buffer at 37 °C (% remaining)

pH	Time (min)				
	1	2	4	8	16
5.8	99	97	95	92	87
6.2	98	96	92	88	76
6.4	96	92	82	66	32
7.0	94	86	75	55	11
7.4	91	80	64	34	0

Dehydroascorbic acid was prepared by bromine oxidation of AA[9] and assayed by the homocysteine method as described in the text.

Table 2. Liver dehydroascorbatase activity in guinea-pigs of different ages

	Age (days)		
	20	250	880
Dehydro-ascorbatase activity*	0.358 ± 0.021 (8)	0.359 ± 0.028 (8)	0.285 ± 0.094 (7)

* Activity expressed as µmoles of DHAA destroyed/g tissue/min (mean values with SE); numbers in brackets refer to the animals in the group.