SPECIALIA

Distribution of Type A and B Monoamine Oxidase Activities in the Central Nervous System of Rat and Chick

O. Suzuki and K. Yagi

Institute of Biochemistry, Faculty of Medicine, University of Nagoya, Nagoya 466 (Japan), 12 May 1975.

Summary. The distribution of type A and B monoamine oxidase (MAO) activities in the central nervous system (CNS) of rat and chick was investigated using 5-hydroxytryptamine and β-phenylethylamine as specific substrates. The distribution of type A MAO was similar to that of type B MAO in rat CNS, but quite different in chick CNS. This may be ascribed to the difference in animal species. The major part of MAO activity in the spinal cord was found to be type A.

Recently, a number of reports have accumulated on multiple forms of monoamine oxidase (MAO) in the brain[1–10]. In these papers, the existence of 2 types of MAO activities, which are designated as type A and B enzymes, has been the focus of interest[1,3,5,7–10]. Even though it is not clear whether each type of MAO has its own enzyme protein, it is unequivocally accepted that substrate specificity and selective inhibition of each type of MAO are demonstrable both in vitro and in vivo. Type A MAO is sensitive to the inhibitor drug, clorgyline; and 5-hydroxytryptamine (5-HT) as well as norepinephrine are preferred substrates for this enzyme. In contrast to type A enzyme, type B MAO is sensitive to the inhibitor drug, deprenyl; and β-phenylethylamine (PEA) is a preferred biogenic substrate.

Although the distribution of MAO activities towards several monoamines in the brain has been reported on dog[11], rat[12] and human[2], the distribution of MAO activity towards PEA, which represents the activity of type B MAO, has never been investigated. The present paper briefly deals with the distribution of MAO activities in the central nervous system (CNS) of rat and chick towards PEA in comparison with those towards 5-HT, as an attempt to know the relationship between the multiplicity and physiological roles of MAO in various regions of CNS.

Male Wistar rats weighing 100–150 g and newborn chicks of the White Rock breed were killed by decapitation. The brain and spinal cord were rapidly removed and kept frozen until analyzed. Frozen rat brain was dissected into 4 parts according to the atlas of Glowinski and Iversen[13], viz. cerebral hemisphere, cerebellum, medulla oblongata plus pons and brainstem. The cerebral hemisphere did not contain the hippocampus. The brainstem included the basal ganglia and hypothalamus. Chick brain was also dissected into 4 parts, viz. cerebral hemisphere, cerebellum, optic lobe and brainstem. The brainstem included the basal ganglia, hypothalamus, medulla oblongata and pons.

MAO activity using 5-HT as substrate was measured according to the method of Karki et al.[14] with slight modification[15]; the concentration of 5-HT in reaction mixture was 0.2 m*M*. The determination of MAO activity using PEA as substrate was carried out by measuring PEA disappearance. The disappearance of PEA was measured by a newly devised method. Inhibition by pargyline (4×10^{-4} *M*), a potent inhibitor of MAO, was used as a criterion of enzyme specificity. The complete inhibition of PEA disappearance by pargyline was observed, indicating thereby the specificity of the assay for MAO activity towards PEA. Tissues were homogenized with 0.9% NaCl in a Potter-Elvehjem homogenizer fitted with a Teflon pestle. The reaction mixture (3.5 ml) contained 20–50 mg of tissue, 0.3 ml of 0.3 *M* sodium phosphate buffer (pH 7.4) and 77 µg of PEA (free base) (final concentration, 0.2 m*M*). The reaction mixture was incubated at 37 °C for 30 min. The reaction was stopped by the addition of 0.2 ml of 60% perchloric acid and the mixture was centrifuged (3,000 rpm, 5 min). 2 ml of the clear supernatant were transferred to a glass centrifuge tube, and 0.5 ml of 5 *N* NaOH and 5 ml of *n*-butanol were added. The mixture was shaken vigorously for 30 sec and centrifuged (3,000 rpm, 5 min). 3 ml of *n*-butanol layer were transferred to another glass centrifuge tube, and 6 ml of *n*-heptane and 1 ml of 0.1 *N* HCl were added. The tube was shaken and centrifuged in the same manner. Subsequently, 0.2 ml of the 0.1 *N* HCl layer was transferred to a test tube, and 0.2 ml of 0.05 *M* ninhydrin solution and 0.5 ml of 0.5 *N* sodium phosphate buffer (pH 8.0) were added. Then, the mixture was incubated at 60 °C for 60 min in an oil bath, cooled with tap water, left at room temperature for 15 min and subjected to the mea-

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Distribution of type A and B MAO activities in CNS of rat and chick

Rat			Chick		
MAO activity (μmoles/g wet weight/30 min)			MAO Activity (μmoles/g wet weight/30 min)		
Regions	Type A (5-HT)	Type B (PEA)	Regions	Type A (5-HT)	Type B (PEA)
Spinal cord	3.18 ± 0.18	0.53 ± 0.16	Spinal cord	6.54 ± 0.47	2.41 ± 0.38
Cerebral hemisphere	4.82 ± 0.46	1.14 ± 0.25	Cerebral hemisphere	2.97 ± 0.47	7.78 ± 0.76
Cerebellum	4.24 ± 0.26	1.17 ± 0.15	Cerebellum	2.51 ± 0.54	3.09 ± 0.60
Medulla oblongata plus pons	4.18 ± 0.21	1.11 ± 0.11	Optic lobe	3.49 ± 0.55	4.52 ± 0.45
Brainstem	5.25 ± 0.51	1.36 ± 0.31	Brainstem	5.33 ± 0.44	10.05 ± 1.39

The concentration of the substrates, 5-HT and PEA, in the reaction mixture for the estimation of type A and B MAO activities was 0.2 m*M*. Means ± SD are given. Each value was obtained from 4 experiments.

surement of its fluorescence with excitation at 390 nm and emission at 490 nm. MAO activity towards PEA was expressed as μmoles of PEA metabolized in 30 min per g fresh tissue. The results are summarized in the Table.

In rat CNS, the reaction rate for PEA was smaller than that for 5-HT, and the pattern of distribution of MAO activity towards PEA was similar to that towards 5-HT. It should be noted that the activities are highest in the brainstem and lowest in the spinal cord. The pattern of distribution of MAO activity towards 5-HT observed in the present study is similar to that reported by HARADA et al.[12].

Differing from the results of rat brain, the activity towards PEA was generally higher than that towards 5-HT in chick brain. It is also noted that the ratio of type A to type B MAO activity is different in various areas of chick CNS. This may not be due to immaturity of CNS of newborn chick during its ontogenic development, but to the difference of animal species, since the metabolism of monoamines in CNS of newborn chick is thought to be well established and similar to that of the adult animal[15,16].

MAO activity towards 5-HT in chick spinal cord was higher than that in any region of chick brain, confirming our previous result[16], whereas the activity towards PEA in chick spinal cord was lower than that in any region of chick brain. Accordingly, a large ratio of type A to type B MAO activity was observed. This is also true in the case of rat spinal cord. These results suggest that MAO in the spinal cord may be associated with the sympathetic nervous system, since MAO in the sympathetic nerves was reported to be almost exclusively type A[17,18].

MAO activity towards PEA in chick cerebral hemisphere was markedly higher than that in any region of CNS with the exception of the brainstem. This should be noted while investigating the physiological significance of type B MAO as well as PEA in the brain.

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Occurrence of Octopamine in the Soft Tissues of the Gastropod Mollusc *Peristernia nassatula* Lamarck[1]

M. ROSEGHINI and A. C. ALCALA

Istituto di Farmacologia Medica I dell' Università, Città Universitaria, I-00185 Roma (Italy), and Department of Biology, Silliman University, Dumaguete City (Philippines), 21 August 1975.

Summary. The soft tissues of the gastropod mollusc *Peristernia nassatula* contain large amounts (30–60 μg/g) of octopamine, which, in all probability, is concentrated in the hypobranchial gland. Smaller amounts of the amine are present also in the soft tissues of the gastropods *Bulla adamsi* and *Latirus polygonatus*.

After its first identification in acetone extracts of the posterior salivary glands of *Octopus vulgaris*[2], octopamine (*p*-hydroxyphenylethanolamine) was detected in the nervous tissue and in other tissues of vertebrates and invertebrates[3–6]. Even human blood seems to contain detectable amounts of the amine[7]. According to SAAVEDRA et al.[8], octopamine may have neurotransmitter function of its own in the central nervous system of molluscs.

This communication describes the occurrence of large amounts of octopamine in extracts of the soft tissues of *Peristernia nassatula* Lamarck, a gastropod mollusc of the family Fasciolaridae.

Materials and methods. Three batches of *Peristernia nassatula* were collected near Dumaguete City (Negros Oriental, Philippines) in November 1973 (139 specimens, 39,1 g), January 1974 (53 specimens, 29,6 g) and July 1974 (197 specimens, 79 g). The whole soft tissues were removed from the living animals after cautious rupture of the shell and immediately extracted with 5 parts (w/v) of pure methanol. After 5–10 days the supernatant liquid was decanted and the tissue re-extracted with 5 parts of