MONOAMINE OXIDATION IN DIFFERENT ORGANS OF RAT

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Summary: Monoamine oxidation has been studied in different organs of adult rats. Activity of monoamine dehydrogenase (MADH) has been measured both aerobically and anaerobically. Brain is the organ where maximum activity of MADH has been observed while in ovary the minimal enzyme activity has been noticed. In the absence of air, the activity proceeded at faster rate whereas MAO activity cannot take place in absence of oxygen. Oxygen can not be replaced by other electron acceptors like NTC. However, in some organs like pancreas, the formazan production was almost negligible. Aerobically moderate MADH activity was observed in case of ileum and testis. On the other hand, spleen, brain, testis and lung homogenates showed moderate amount of tetrazolium salts reduction in absence of air. This indicates the differential nature of the MADH activities in aerobic and anaerobic condition. High activity of monoanine oxidase (MAO) has been observed in liver. Brain and pancreas were also found good organs for MAO activity, but liver homogenate failed to reduce tetrazolium salt. Only dialysed liver homogenate in the presence of tryptamine, demonstrated moderate activity of MADH. Relative activity of both the enzymes has been studied. The organwise distribution pattern of MAO and MADH appeared quite different.

Key words: rat organs monoamine oxidation MAO MADH

INTRODUCTION

Monoamine oxidase (MAO) (monoamine O_2 oxido reductase, deaminating, EC 1.4.3.4.) is a ubiquitous enzyme occuring in all classes of vertebrate tissues and in many invertebrtes (1). It is believed to be the enzyme which oxidises naturally occuring phenylethylamines, dopamine, noradrenaline, serotonin, etc. (15,17) inside the body. There are several reports which demonstrate the distributional pattern of this enzyme in different tissues and organs of various species including man (10, 13, and 14). It is thought that there are two distinct enzymes with different pH maxima present as a mixture in tissues and are equally responsible for the breakdown of biologically active monoamines (18). This concept is further supported by the kinetic studies on the inhibition of the oxidation of serotonin and tyramine by rat liver mitochondria (9). Our recent observations (4, 7 and 8) strongly suggest that besides MAO, there is another enzyme, monoamine dehydrogenase (MADH) which is capable of degrading biogenic amines by the process of dehydrogenation. With this in view, the present work has been designed to find out the distributional pattern of these two enzymes, namely MAO and MADH in different organs of rat and to see whether the distribution pattern of these two enzymes remains same or different.

MATERIALS AND METHODS

Enzyme preparations:

Adult albino rats of body weight 150-200 g were used throughout the experiment. The rats were killed by decapitation and the organs were removed, weighed and placed in nine volumes of ice cold 0.25 M source to obtain 10 per cent suspension. Each of the organs was homogenized

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in a Potter-Elvehjem homogenizer. All subsequent tissue peparations were carried out in ice-cold condition and incubated as described below. Incubations were carried out at 38° for 30 minutes in case of MAO and MADH (aerobic) while the same period was reduced for 15 min only in case of MADH (anaerobic) assay method.

Assay of MAO activity:

The standard incubation mixture consisted of 0.02 M phosphate buffer pH 6.5, 0.0125 M semicarbazide pH 6.5, 0.01 M tyramine and 50 *mg* of tissue homogenate in a final volume of 2 *ml*. The enzyme activity was determined by measuring the aldehyde formation in a Bausch and Lomb Colorimeter at 420 nm by the method as described previously (8).

Assay of MADH activity :

The reaction mixture for MADH assay contained 0.025 M phosphate buffer pH 7.0,0.5 *mg* neo-tetrazolium chloride (NTC), 0.01 M tyramine and 100 *mg* of tissue homogenate in a final volume of 2 *ml*. NTC reduction was measured at 520 *nm* in a Bausch & Lomb colorimeter according to the method of Lagnado and Sourkes (12). Air was used as the gas phase in aerobic experiments and anaerobic experiments were carried out *in vacuo* in Thunberg tubes.

Enzyme units:

The unit of MAO activity was expressed in terms fo products i.e. in terms of 2:4-dinitrophenylhydrazones of p-hydroxyphenyl-acetaldehyde formed per minute calculated from a calibration curve made with a twice crystallised derivative prepared according to Guha and Ghosh (8).

MADH unit was expressed as *μmoles* of diformazan formed per minute calculated from a calibration curve obtained with a commercial product of Nutritional Biochemical Corporation, U.S.A.

Total enzyme units in the homogenate and different organs were determined by multiplying enzyme units present per *ml* of the corresponding preparations with the respective volumes.

RESULTS AND DISCUSSION

Table I shows the activities of MAO and MADH (aerobic and anaerobic) in various organs of adult rat. Highest MADH (aerobic) activity (either per g tissue or units per organ) was observed in brain, while moderate activity was found in organs like ileum, testis, spleen etc. and lowest activity was noticed in ovary.

Anaerobically, no activity of MADH could be detected from heart tissue due to high reduction of NTC by the endogenous substrates. Usually, the activity of MADH per g tissue under an aerobic conditions became double as it was found with brain. But it did not hold good with all the tissues, for instance, ovary (approximately, four times of aerobic value), kidney (approximately five times of aerobic value), etc. Anaerobically, spleen contained highest quantity of MADH (per g tissue) followed by brain, while ovary possessed lowest MADH activity either per g tissue or units per organ. When total enzyme activity was considered, brain, showed maximal MADH activity followed by spleen. This definitely shows that anaerobic activity of MADH differs from that of aerobic activity.

TABLE I: MADH and MAO activities in different organs of adult rat.

Sl. No.	Different organs	MADH activity (μ moles diformazan formed/min.) Aerobic		MADH activity (μ moles diformazan formed/min.) Anaerobic		MAO activity μmoles of 2:4 DNP hydrazone of p-hydroxyphenylacetaldehyde formed/min)	
		Per g tissue (Mean±S.D.)	Whole organ (Mean±S.D.)	Per g tissue (Mean±S.D.)	Whole organ (Mean±S.D.)	Per g tissue (Mean±S.D.)	Whole organ (Mean±S.D.)
1.	Ovary	0.62±0.11 (15)	0.031±0.006	2.50±0.43 (30)	1.25±0.19	5.56±0.173 (25)	0.278±0.079
2.	Testis	1.87±0.25 (45)	1.029±0.130	4.58±0.61 (55)	2.519±0.35	17.22±3.66 (77.7)	9.471±1.861
3.	Spleen	1.46±0.31 (35)	0.730±0.162	8.54±1.87 (102.9)	4.27±0.76	9.44±2.42 (42.5)	4.72±1.21
4.	Kidney	0.83±0.23 (20)	0.273±0.078	4.17±1.19 (50)	1.352±0.38	13.18±2.53 (62.5)	4.511±0.867
5.	Ileum	2.08±0.42 (50)	3.90±1.14	5.00±0.89 (60)	9.10±1.56	17.22±4.92 (70.5)	31.98±9.69
6.	Duodenum	1.56±0.36 (37.5)	0.234±0.041	4.17±0.92 (50)	0.624±0.17	15.00±2.32 (67.5)	2.25±0.398
7.	Liver	—	—	—	—	23.32±5.06 (105)	74.624±16.583
8.	Heart	2.29±0.71 (55)	0.630±0.196	Nil	Nil	11.66±4.02 (52.5)	3.262±1.019
9.	Lung	1.66±0.32 (40)	1.660±0.319	4.58±0.80 (55)	4.58±0.81	15.00±3.13 (67.5)	15.00±3.132
10.	Pancreas	Nil	Nil	Nil	Nil	22.22±3.89 (100)	19.998±3.571
11.	Brain	4.16±1.18 (100)	6.240±1.782	8.32±1.39 (100)	12.480±1.73	22.22±4.83 (100)	33.330±9.502

Figures in parentheses indicates relative activity of the enzyme.

Pancreas was the tissue where MADH activity (both aerobic and anaerobic) was very negligible. Like rat pancreas, guinea pig vas deference was a devoid of MADH activity. MADH activity in dialysed liver homogenate operated better; without dialysis, it appeared very difficult to show NTC reduction even in different systems (Table II). Dialysed liver homogenate with tryptamine as substrate demonstrated the presence of MADH, while that with tyramine low activity was observed. Same type of observation was reported with liver homogenate for the determination of succinic dehydrogenase activity with the help of NTC.

In case of MAO activity, again, ovary possessed lowest activity either per g tissue or total units. Liver contained maximal activity of MAO (both per g tissue and total units). Pancreas and brain were the other organs which showed high ativity of MAO. However, organwise distribution of MAO activity appeared quite different from that of MADH, especially from the

aerobic MADH activity. Again Table III indicates that the rabbit ileum contained very high activity of MAO while MADH (aerobic) activity was comparatively lower.

TABLE II: NTC reduction with rat liver homogenate.

Sl. No.	System	NTC concentration	Substrate used	*MADH activity (μ moles of diformazan formed/min/g/tissue) Aerobic	Anaerobic
1.	Liver homogenate 100 *mg* tissue/test tube or 120 *mg* tissue/test tube	0.5 *mg* 1.0 *mg*	Tyramine 1 x 10^{-2}M	Nil	Nil
2.	Liver homogenate 100 *mg* tissue/test tube or 120 *mg* tissue/test tube	0.5 *mg* 1.0 *mg*	Tryptamine 6 x 10^{-3}M	Nil	Nil
3.	20 per cent liver homogenate dialysed against water for 15 hrs at 2°C				
	100 *mg* tissue/test tube	0.5 *mg*	Tryptamine 6 x 10^{-3}M	2.7±0.51	Nil
	120 *mg* tissue/test tube	1.0 *mg*	Tryptamine 6 x 10^{-3}M	3.8±1.11	Nil
4.	20 per cent liver homogenate dialysed against water for 15 hrs. at 20°C.				
	100 *mg* tissue/test tube	0.5 *mg*	Tyramine 1 x 10^{-2}M	1.1±0.19	Nil
	120 *mg* tissue/test tube	1.0 *mg*	Tyramine 1 x 10^{-2}M	1.7±0.22	Nil

*Mean±S.D.

TABLE III: MAO and MADH (aerobic) activities of rabbit ileum.

Sl. No.	*MAO activity (μ moles of 2:4 DNP hydrazone of p-hydroxyphenylacetaldehyde formed/min.) Per g tissue	*MAO activity Whole tissue	*MADH (aerobic) activity (μ moles of diformazan formed/min) Per g tissue	*MADH (aerobic) activity Whole tissue
1.	250±32.05	1125±114.23	3.33±0.95	14.985±4.282

*Mean ±S.D.

In the case of MAO and MADH, the relative activities of the ileum and duodenum are more or less same, although a comparatively low activity was observed in the duodenum. It is reported that the low MAO activity in the intestine protects serotonin from its further degradation and this may be reflected by the high level of serotonin known to be present in the gastrointestinal tract as described in the literature. (14). However, the serotonin level in tissues and the level of the concerned enzymes do not show any positive correlation and this may be partly related to the different subcellular location of these enzymes. High content of MADH and MAO in the testis than the female gonad attracts special attention. This may indicate that biogenic amines take part a number of physiological functions including gametogenesis. It is also reported that the activity of MAO from different tissues depends upon the substrate used. As in the present study, tyramine was used as substrate, so the present results do not necessarily follow the observations of the previous authors (13).

The present findings further support the idea that MADH is a differnt enzyme. Recently, Guha and Ghosh (8) based on the results obtained from *in vitro* and *in vivo* behaviour of inhibitors and reaction mechanism, have suggested that MADH is possibly different from MAO, They have clearly shown that MAO requires oxgen for its reaction mechanism. While MADH activity proceeds at faster rates in anerobic condition and oxygen cannot be replaced by other clectron acceptor like NTC. However, reports ate now available on the electrophoretic separations of MAO isoenzymes where enzyme is assayed with tetrazolium salts (nitroblue tetrazolium) like histochemical assay techniques of MAO demonstration (5), but it is found liable to artifacts and its value for actual monoamine oxidase detection appears doubtful (2). Again, detailed kinetic studies on these two enzymes behave quite differently from each other (4). The concept, relating to oxidative deamination of monoamines by more than one enzyme (6, 16) is further strengthened from the present investigation that MADH is possibly an another enzyme which takes part in the physiological inactivation of biogenic amines, of course, further work in this direction appears essential, especially to elucidate its actual role in connection with monoamine metabolism.

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