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EFFECT OF OXYGEN TENSION ON MONOAMINE OXIDASE ACTIVITY

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Abstract—Increased oxygen tension increases the activity of monoamine oxidase (MAO) obtained from various tissues of rats, mice, and guinea pigs. The increase varies with tissue, species, and substrate. Per cent inhibition of MAO by iproniazid, *d,l*-amphetamine, and trans-2-phenylcyclopropylamine is not changed by altered oxygen tension. Finally, the response of the isolated right atrium of the rat to tyramine is altered by the oxygen tension of the bath. This effect can be explained on the basis of the change in intracellular MAO activity.

MONOAMINE oxidase (MAO) activity increases with an increase in the oxygen tension of the assay environment.[1, 2] This property of MAO has been relatively neglected, and most laboratories use either air or 100% O_2 for MAO assay. We have investigated the relationship of O_2 tension and MAO activity, using various tissues and species as sources of MAO.

METHODS

Assays in vitro. Liver and heart mitochondria were isolated according to Schneider,[3] and brain mitochondria were isolated according to Abood and Gerard.[4] MAO activity was measured as the rate of O_2 uptake in the Warburg apparatus. The reaction mixture consisted of substrate, mitochondria from 250–300 mg wet weight tissue, and 20 μm semicarbazide contained in a final volume of 2·5 ml Na-phosphate buffer (0·067 M, pH 7·4). The temperature was maintained at 37°.

Substrates were tyramine HCl, 5 hydroxytryptamine-creatine SO_4, tryptamine, and β-phenylethylamine. Substrate concentration varied from 6–8 × 10^{-3} M dependent upon the substrate used. MAO activity was calculated from the O_2 uptake during the initial 15 min after addition of substrate for liver assays and 20 min for heart and brain assays.

Gas phase was varied by using air or flushing the reaction flask with the desired O_2-N_2 mixture for at least 5 min at 37° prior to temperature equilibration and addition of substrate.

Isolated tissue assay. Male Wistar rats (300–400 g) were killed by decapitation and the heart removed immediately and placed in oxygenated Tyrode's solution at 37°. The right atrium was removed and tied between a Sanborn transducer and a stationary glass rod at the bottom of the tissue bath (50 ml). After 15 min for equilibration, a control spontaneous heart rate was recorded, then tyramine was added to the bath in 0·1 ml Tyrode's solution so that the final bath concentration was 10 μg/ml. The peak

response of increased heart rate was recorded. Each heart preparation was assayed twice with 95% O_2–5% CO_2 or air to oxygenate the bath solution.

RESULTS AND DISCUSSION

Specificity of MAO *assay* in vitro

The specificity of the assay was shown by the 100 per cent inhibition of O_2 uptake with either iproniazid (2×10^{-4} M) or 2-phenylcyclopropylamine (2×10^{-5} M), with 8×10^{-3} M tyramine as the substrate. Ammonia production was compared to O_2 consumption,[5, 6] and there was a stoichiometric agreement between O_2 consumption and NH_3 production with tyramine as the substrate and either air or 100% O_2 as the gas phase. Liver mitochondria were diluted with buffer so that samples contained 25%, 50%, or 75% of the original preparation. These samples were assayed with tyramine as substrate in air or 100% O_2. The expected straight-line relationship was obtained when activity was plotted against enzyme concentration.

O_2 *Tension and* MAO *activity*

Rat liver mitochondria were assayed for MAO activity under different O_2 tensions with tyramine or 5-hydroxytryptamine as substrate. The O_2 uptake was measured for the initial 15 min and the results expressed as microliters O_2 uptake per gram fresh weight tissue per 30 min. Each assay was run in triplicate with mitochondria from 250 mg wet weight tissue in each flask. Both substrates were oxidized at similar rates under O_2 tensions of 20% or below; however, the rate of tyramine oxidation was 3·1 times that of 5-hydroxytryptamine in 100% O_2 (Fig. 1).

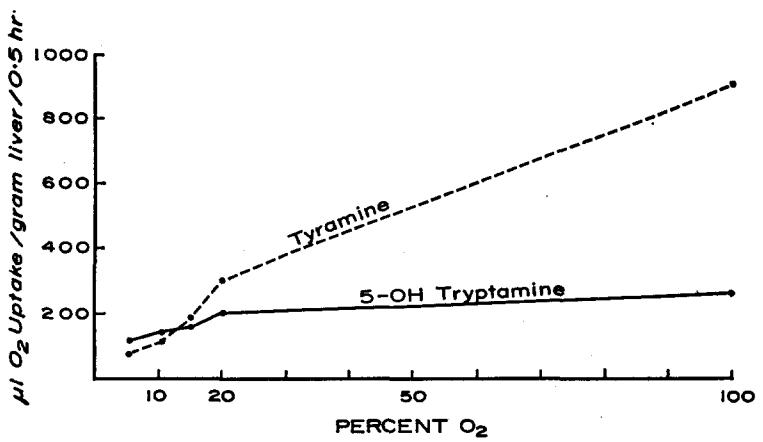

FIG. 1. MAO activity of rat liver mitochondria assayed at various oxygen tensions. Each point is the average of three determinations (mitochondria from 250 mg fresh weight tissue).

Since the largest differences in rate occurred between air and 100% O_2, these two gas phases were used in the assay of liver MAO from rats and guinea pigs (Table 1). The "O_2 effect" (rate in oxygen divided by rate in air) varied from 1·4 to 3·3, dependent upon the species and substrate.

Table 2 shows that the "oxygen effect" varied considerably, dependent upon the tissue, species, and substrate. The only relatively consistent effect was obtained with guinea pig liver which had a small oxygen effect regardless of the substrate.

Inhibition of MAO

Rat liver mitochondria were assayed for MAO activity in air or 100% O_2 in the presence of three MAO inhibitors—iproniazid, *d,l*-amphetamine, and trans-2-phenylcyclopropylamine. Tyramine was used as the substrate. The per cent inhibition did not vary with the oxygen tension (Table 3).

TABLE 1. EFFECT OF O_2 TENSION ON HEPATIC MAO ACTIVITY IN RATS AND GUINEA PIGS AS MEASURED BY O_2 UPTAKE

Species	No. of assays	Substrate (8×10^{-3} M)	Air (μl/250 mg/15′)	O_2 (μl/250 mg/15′)	Rate in O_2/ rate in air
Rat	25	Tyramine	32·2 ± 3·5*	106·8 ± 9·0*	3·3
Rat	17	5-Hydroxytryptamine	22·8 ± 4·0	31·2 ± 4·0	1·4
Rat	4	Tryptamine	14·9 ± 3·2	30·9 ± 6·1	2·1
Guinea pig	4	Tyramine	36·7 ± 1·5	73·5 ± 3·0	2·0
Guinea pig	4	5-Hydroxytryptamine	35·8 ± 2·1	52·1 ± 7·4	1·4

* Standard deviation.
Mitochondria from 250 mg wet weight tissue were used as source of enzyme.

TABLE 2. EFFECT OF O_2 TENSION ON RATE OF MAO

Species	Tissue	Rate of O_2/rate in air			
		Tyramine	5-Hydroxy tryptamine	Tryptamine	β-Phenyl-ethylamine
Rat	Liver	3·3	1·4	2·1	5·1
	Heart	2·0	1·3	1·5	6·0
	Brain	2·1	1·2	1.1	1·1
Mouse	Liver	4·2	1·9	3·8	6·0
Guinea pig	Liver	2·0	1·4	1·4	1·4
	Heart	2·3			

Oxygen effect was calculated as the rate in 100% O_2 divided by the rate in air. Liver assays were run on mitochondria from 250 mg wet weight tissue; brain and heart assays were run on mitochondria from 300 mg wet weight tissue.

TABLE 3. INHIBITION OF RAT LIVER MAO ASSAYED IN AIR OR 100% O_2

Inhibitor	Concentration (M)	% Inhibition	
		Air	100% O_2
d,l-Amphetamine	2×10^{-3}	60	66
Iproniazid	2×10^{-5}	32	39
2-Phenylcyclopropylamine	2×10^{-6}	55	52

Tyramine (8×10^{-3} M) was used as substrate and mitochondria from 250 mg wet weight rat liver as the source of enzyme. The same supply of mitochondria was used for all assays.

Additional studies in vitro

Various procedures were tried in an attempt to alter the oxygen effect. Tyramine was used as the substrate and rat liver as the source of MAO. Incubation of enzyme in 100% O_2 for 10 min followed by the estimation of MAO in air resulted in a rate

equal to air incubation alone. Heating mitochondria for 20 min at 48–50° reduced MAO activity by 50% but failed to alter the O_2 effect, nor did disruption of mitochondria by snake venom, sonic vibration, or osmotic changes, all of which reduced MAO activity, have any effect. Although rat heart MAO activity increased with age[7] and thyroid administration, there was no alteration in relative O_2 effect. It appears

TABLE 4. RESPONSE OF RAT ISOLATED RIGHT ATRIUM TO TYRAMINE IN A 50-ml BATH OF TYRODE'S SOLUTION AT 37°

Average body weight (g)	Pretreatment	No. of animals	Control heart rate air	Increase in heart rate air and 10 μg tyramine/ml	Control heart rate 95% O_2–5% CO_2	Increase in heart rate 95% O_2–5% CO_2 10 μg tyramine/ml
426	None	7	239	+ 106 ± 41*	233	+ 61 ± 22†
450	Iproniazid, 50 mg/kg, i.p. 16 hr before assay	8			268	+ 128 ± 37

* $P < 0{\cdot}05$ compared to rate in O_2, not significant to iproniazid-treated group.
† $P < 0{\cdot}01$ compared to iproniazid-treated group.

that the sensitivity of MAO to O_2 tension is an inherent property of the enzyme. This should be considered in all studies where kinetics or relative activities are determined.

Isolated tissue study

In an attempt to demonstrate the effect of O_2 tension on MAO activity other than an enzyme assay, the response of the isolated rat right atrium to tyramine was studied in air and 95% O_2–5% CO_2. Tyramine-induced tachycardia is markedly potentiated by MAO inhibition. The level of this response is determined by the activity of MAO within the isolated atria.[8] Low MAO activity in air compared to 95% O_2–5% CO_2 could then be considered equivalent to partial MAO inhibition. It was found that tachycardia after tyramine was significantly greater when assayed in air then when assayed in 95% O_2–5% CO_2 (Table 4). The response in air was only slightly below that observed when MAO was inhibited by iproniazid and assayed for tyramine response in 95% O_2–5% CO_2. This suggests that the sensitivity of MAO to O_2 tension occurs in the intact cell as well as in isolated mitochondria.

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