THE OXIDATION OF MESCALINE AND CERTAIN OTHER AMINES*

By FREDERICK BERNHEIM and MARY L. C. BERNHEIM

(From the Departments of Physiology and Pharmacology and Biochemistry, Duke University School of Medicine, Durham, North Carolina)

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Mescaline, 3,4,5-trimethoxyphenylethylamine, has been shown by Slotta and Müller (1) to be excreted in the urine in the form of the corresponding acid when it is fed to various animals. It was therefore of interest to study the oxidation of mescaline *in vitro* and compare it with that of tyramine and other amines such as β-phenylethylamine, β-phenyl-β-oxyethylamine, and isoamylamine which are oxidized by the tyramine oxidase. The results showed that the introduction of methoxy groups into the tyramine molecule definitely affected the properties of the oxidation. Mescaline is the active substance of a Mexican cactus (*Anhalonium lewinii*) and produces color visions and other effects when taken internally. The oxidation of mescaline which takes place in the liver is therefore in all probability a detoxicating mechanism.

EXPERIMENTAL

An active tyramine oxidase is found in the liver and kidney of the rat, guinea pig, cat, dog, and rabbit as well as in other animals which were not used in this investigation. Under optimal conditions for the oxidation of tyramine, the oxidation of mescaline was investigated in the tissues of the various animals. The liver or kidneys were chopped with scissors, ground with sand in 0.05 M phosphate buffer at pH 7.8 (approximately 1.0 cc. of buffer per gm. of tissue), and pressed through muslin. Rabbit liver oxidizes mescaline rapidly, rat and guinea pig liver and rabbit kidney very slowly, and rat and guinea pig kidney and cat and dog

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liver and kidney not at all. For the further study of the oxidation the rabbit liver was used. A standard preparation was made by dialyzing the preparation made as above against distilled water until the preparation alone had almost no oxygen uptake. The liver of an adult 2 kilo rabbit yielded about 150 cc. of the dialyzed suspension. 1.0 cc. of this preparation at pH 7.8 and 37° can oxidize 1.0 mg. of mescaline sulfate (Hoffmann-La Roche) to 3,4,5-trimethoxyphenylacetic acid in 2 hours with the uptake of 80 c.mm. of oxygen.

If the liver suspension is centrifuged, a brown precipitate comes down leaving a cloudy solution which contains most of the hemoglobin. Both fractions have about equal activity in oxidizing tyramine and mescaline. The precipitate can be washed free of hemoglobin with phosphate buffer of pH 6.7 and centrifuged and finally suspended in buffer of pH 7.8 and this procedure causes only a slight loss of activity compared with that of the original precipitate, which represents, of course, less than 50 per cent of the activity of the original suspension. Except for certain cases, therefore, the original dialyzed suspension was used.

As stated above, the rabbit kidney oxidizes mescaline slowly. If the kidney suspension is dialyzed and centrifuged, the solid contains a very active tyramine oxidase but mescaline is not oxidized. The liquid which is cloudy and contains the hemoglobin oxidized tyramine more slowly but oxidized mescaline at about the same rate as the original suspension. For the purposes of comparing the activity of the solid and the liquid after centrifuging, the solid was made up to a volume equal to the liquid by the addition of phosphate buffer at pH 7.8. To sum up these facts, it is possible to get preparations that will oxidize tyramine but not mescaline but so far no preparation has been obtained that will oxidize mescaline but not tyramine. Certain other amines, β-phenylethylamine, β-phenyl-β-oxyethylamine, and isoamylamine, are oxidized by all the preparations that oxidize tyramine.

In order to determine whether it was possible to separate the mescaline oxidation from the tyramine in rabbit liver the following experiments were carried out. The relative rates of tyramine and mescaline oxidation were measured after dialyzing for varying lengths of time and compared with a suitably diluted undialyzed control. Both oxidation rates decreased slightly with time but

to the same extent. Liver suspension which was stirred into 20 volumes of water formed a precipitate which settled out and was washed with water several times. This preparation was active in oxidizing both tyramine and mescaline but was unable to oxidize succinate, amino acids, choline, and lactate. A liver preparation was allowed to autolyze at 37° for 48 hours with toluene as a preservative, and the rates of oxidation for both tyramine and mescaline decreased equally. Finally, heating the preparation at different temperatures for varying lengths of time caused an equal loss in oxidation rates of both substances.

It was possible that the inability of certain tissues to oxidize mescaline might be caused by the fact that the affinity constant of the enzyme for mescaline was less than that for tyramine, but this is not the case. Kohn (2) has measured the affinity constant for tyramine using a purified pig liver preparation. He obtained a half saturation value with a tyramine concentration of 0.5×10^{-3} M, and a saturation value of 2.5×10^{-3} M. We have been able to confirm this for the rabbit liver preparation and have been able to show that the saturation value for mescaline is 0.25×10^{-3} M; in other words, its affinity constant is greater than that for tyramine. No accurate half saturation values could be obtained.

Bernheim (3) showed that tyramine took up 1, 2, or 4 atoms of oxygen per molecule depending on the age and treatment of the tissue used. Kohn (2) was able to purify the enzyme so that only 1 atom was taken up. The rabbit preparation used in these experiments causes tyramine to take up 1 atom of oxygen very rapidly; the slope of the curve then abruptly changes and the uptake of the 2nd atom takes place more slowly and usually not more than 30 per cent of the 2nd atom is taken up during the experimental period. Mescaline, however, takes up 2 atoms and there is no change in the slope of the curve after the 1st atom of oxygen has been taken up. The oxidation of several other amines was therefore investigated. The results showed that the same preparation under the same conditions caused an uptake of 2 atoms of oxygen in the case of β-phenylethylamine and β-phenyl-β-oxyethylamine with no change in slope. With isoamylamine it is necessary to have the correct enzyme-substrate relationship, which had to be determined for each preparation. If too little

enzyme was present in relation to the substrate, the oxidation ended abruptly before the theoretical uptake for 1 atom was reached. If too much enzyme was present, there was a tendency for somewhat more than 1 atom to be taken up. But in a small but definite range of concentration exactly 1 atom of oxygen was utilized for every molecule of isoamylamine. Fig. 1 shows the experimental oxygen uptakes in relation to the theoretical uptakes for all five amines and also shows their relative oxidation rates when saturation concentrations were used in all cases. The relative positions of the curves are not changed by using the amines in amounts which in each case will give an uptake of 100 c.mm. of oxygen. Hordenine, putrescine, and cadaverine are attacked slowly by the liver preparation but their oxidation rates are too slow for the determination of a definite end-point.

The fact that mescaline takes up 2 atoms of oxygen indicates that the corresponding acid is formed. In order to prove this 100 mg. of mescaline sulfate were oxidized by 50 cc. of the dialyzed rabbit liver preparation at pH 7.8. At the end of the oxidation a small amount of acetic acid was added until the proteins precipitated. The flask was then put in boiling water for 5 minutes and the coagulum filtered off. The filtrate was evaporated to about 10 cc. at room temperature under a fan. Enough hydrochloric acid was then added to make the solution about 0.2 N and any precipitate which formed was filtered off. The solution was then thoroughly extracted with ether. Occasionally when the ether evaporated off, crystals were left. Usually, however, an oil remained which was dissolved in the minimal amount of benzene. To this an excess of low boiling petroleum ether was added, the mixture was cooled, and the crystals filtered off. Recrystallization was carried out in the same way. Light yellow crystals were obtained which melted at 116°, uncorrected. The analysis was, theoretical, C 58.45, H 6.2; found, C 58.82, H 6.3. The yield was 56 per cent.

Hare (4) has shown that oxidation of tyramine is insensitive to cyanide during the uptake of 1 atom of oxygen per molecule but that the uptake of the 2nd atom was cyanide-sensitive (3). Mescaline takes up 2 atoms of oxygen per molecule, so cyanide was added to see whether the uptake of the 2nd atom was cyanide-sensitive. Surprisingly, cyanide completely inhibited the oxygen

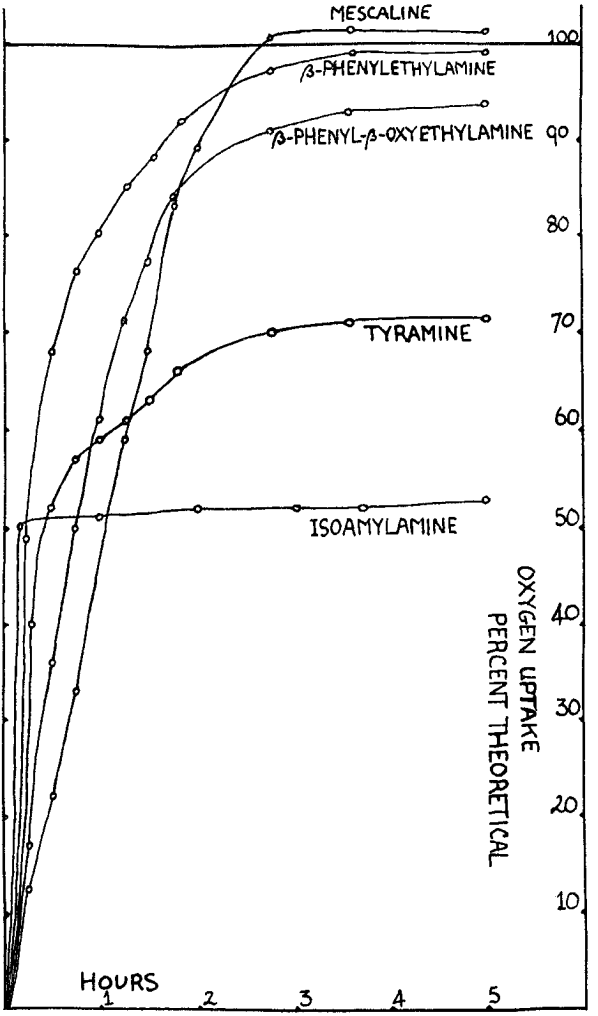

FIG. 1. The oxidation of the five amines by the rabbit liver preparation at pH 7.8. The oxygen uptakes are in per cent of the theoretical, assuming the utilization of 2 atoms of oxygen per molecule of amine. 1.0 mg. of each amine was used.

uptake in concentrations that had no effect on the uptake of the 1st atom of oxygen by tyramine in the same preparation. Thus 0.01 M KCN had no effect on the oxidation of tyramine, β-phenyl-

ethylamine, β-phenyl-β-oxyethylamine, and isoamylamine but completely inhibited that of mescaline. In order to confirm this a cyanide concentration curve was plotted and the results are shown in Fig. 2. KOH was not used in the vessels, so that in each

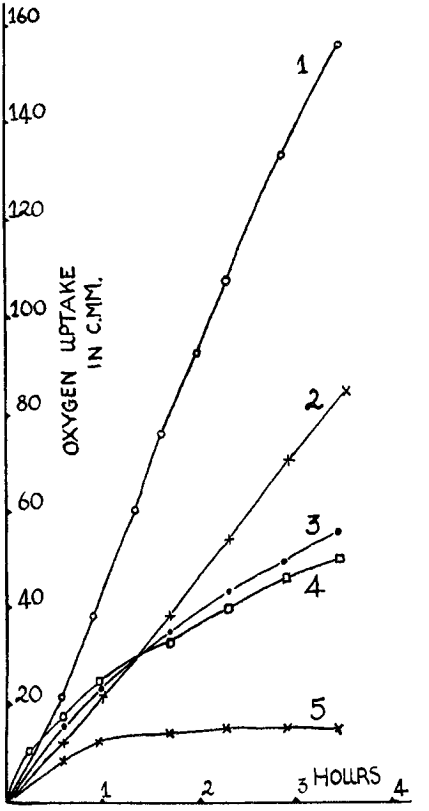

FIG. 2. The effect of various drugs on the oxidation of 2.0 mg. of mescaline sulfate by the rabbit liver preparation at pH 7.8. Curve 1 control, Curve 2 with 0.001 M potassium cyanide, Curve 3 with 0.008 M sodium borate, Curve 4 with 0.1 M potassium pyrophosphate, Curve 5 with 0.005 M potassium cyanide.

case the original cyanide concentration remained constant except for losses due to its oxidation. As the concentration was lowered, the inhibition became less until it was only about 40 per cent when 0.001 M KCN was used. A true cyanide-sensitive system

should be inhibited almost completely with a concentration of 0.001 M KCN. The indication here is that cyanide may be displacing the mescaline from the surface of the enzyme. That the enzyme belongs to the cyanide-insensitive group is indicated by the methemoglobin formation by H_2O_2 when hemoglobin is added. Bernheim and Michel (5) have shown that methemoglobin is only formed under these conditions when cyanide-insensitive systems are used and mescaline produces as much methemoglobin as tyramine does. In a typical experiment when tyramine and mescaline had taken up the same amount of oxygen, 14.0 mg. of extra methemoglobin were formed by the tyramine and 13.5 mg. by the mescaline. The cyanide inhibition is obtained whether the cyanide is added to the enzyme before the mescaline or after it; in other words, the presence of the substrate does not protect the enzyme from the cyanide effect in the sense that xanthine protects xanthine oxidase from it (6). Fig. 2 shows that 0.1 M pyrophosphate and 0.008 M borate also inhibit the mescaline oxidation. In these concentrations the two drugs have no effect on the oxidation of tyramine or of the other three amines. The inhibitory effect of borate on mescaline makes it impossible to plot an accurate pH curve in the alkaline range. As in the case of tyramine, the uptake of the 2nd atom of oxygen by β-phenylethylamine and β-phenyl-β-oxyethylamine is inhibited by 0.005 M KCN. With lower concentrations the inhibition is incomplete.

Kohn (2) made the interesting discovery that the rate of oxidation of tyramine was increased by increasing the oxygen tension. We have been able to confirm this and have tested the effect of increased oxygen tension on the rate of oxidation of the other amines. Table I shows the results. The oxidation rates of β-phenylethylamine and β-phenyl-β-oxyethylamine and mescaline are increased by raising the oxygen tension from 20 per cent to 100 per cent. Hordenine, putrescine, and cadaverine are only slightly affected by increase in oxygen tension. Isoamylamine again shows a peculiarity. Increased oxygen tension increases the rate of oxidation but definitely depresses the amount of oxygen taken up, so that the oxidation stops sharply at less than 1 atom but not at any definite fraction of 1 atom. The explanation for this and also for the sensitivity of the oxidation to the correct enzyme-substrate relationship probably lies in the toxicity of the

end-product for the enzyme. Oxygen itself is not toxic to the enzyme, because it is possible to shake it in 100 per cent oxygen for at least 2 hours before adding the substrate without a decrease in its activity.

The ammonia produced by the deamination of the mescaline and the other amines was estimated by a vacuum distillation method and nesslerization after the oxidation was complete. The recoveries of ammonia in per cent of the theoretical were as follows: mescaline, 94 per cent; β-phenylethylamine, 97 per cent;

TABLE I

Typical Experiment Showing Effect of Oxygen Tension on Rate of Oxidation of Various Amines by Rabbit Liver Preparation, pH 7.8

Amine	O_2 uptake		Increase	Time
	20 per cent O_2	100 per cent O_2		
	c.mm.	*c.mm.*	*per cent*	*min.*
Tyramine	14	66	370	5
	56	137	145	15
Mescaline	4	14	250	5
	20	35	75	15
β-Phenylethylamine	18	84	366	5
	54	187	264	15
β-Phenyl-β-oxyethylamine	6	28	366	5
	21	61	191	15
Isoamylamine	31	63	204	5
	89	113	27	15
	179	132	−26	35
	225	134	−40	60

β-phenyl-β-oxyethylamine, 92 per cent; isoamylamine, 108 per cent; tyramine, 72 per cent. The figures are the average of at least two determinations. All except tyramine show satisfactory agreement with the theoretical. The tyramine yields were consistently low, and the reason for this discrepancy has not been determined.

DISCUSSION

The introduction of methoxy groups into the benzene ring of tyramine definitely modifies the molecule in respect both to its

oxidative deamination by tissues and its pharmacological action. In comparison with tyramine the oxidation rate of mescaline is slow but the corresponding acid is formed by preparations which are able to form only small amounts of the acid from tyramine. The two phenylethylamines are also oxidized to their corresponding acids. These facts suggest that a free hydroxy group in the para position inhibits the oxidation of the aldehyde group formed after deamination, but has no effect, as the relative oxidation rates of tyramine and β-phenylethylamine show, on the deamination itself. The rate of deamination is, however, affected not only by methoxy groups in the ring but also by a hydroxy group in the β position as the relative oxidation rates of β-phenylethylamine and β-phenyl-β-oxyethylamine show.

The fact that only the tyramine oxidase preparation of rabbit liver is able adequately to oxidize mescaline under the conditions of our experiments indicates that some factor other than the oxidase itself is necessary. This is also indicated by the fact that cyanide, pyrophosphate, and borate inhibit the mescaline oxidation in concentrations that leave the oxidation of tyramine and the other amines unaffected. Since all these substances form complexes with heavy metals, it is possible that some heavy metal is necessary with the tyramine oxidase to effect the oxidation of mescaline. This would be different from the cytochrome-indophenol oxidase system, for the latter is inactivated by prolonged dialysis and extensive washing as shown by the inability of the preparations to oxidize succinate. It is also thermolabile, because addition of boiled rabbit liver extracts to tyramine oxidase of other tissues does not enable them to oxidize mescaline.

It has been shown by Dakin (7) that methyl tyrosine is apparently metabolized differently from tyrosine itself. The oxidation of mescaline when compared with that of tyramine shows that methoxy groups in the benzene ring of amines closely related to tyrosine can affect the rate and course of the oxidation of these compounds *in vitro*.

SUMMARY

1. The oxidation of mescaline by a rabbit liver preparation has been described. This preparation contains an active tyramine oxidase but the tyramine oxidase in the tissues of several other animals is unable to oxidize mescaline.

2. Mescaline is oxidized to the corresponding acid which has been isolated. The theoretical amount of ammonia was recovered.

3. Unlike the oxidation of tyramine, the oxidation of mescaline is inhibited by pyrophosphate, borate, and relatively large concentrations of cyanide. Like that of tyramine the oxidation rate of mescaline is increased by raising the oxygen tension. Hydrogen peroxide is produced during the oxidation.

4. The properties of the oxidation of β-phenylethylamine, β-phenyl-β-oxyethylamine, and isoamylamine are similar to those of tyramine.

Addendum—Since this paper was submitted, Richter (8), Blaschko, Richter, and Schlossmann (9), and Pugh and Quastel (10) have published results on the oxidation of tyramine and other amines. Richter has isolated aldehydes after the oxidation of various amines. Blaschko *et al.* have studied the specificity and distribution of the enzyme and identified it with "adrenalin oxidase." See Blaschko, Richter, and Schlossmann for reference to Blaschko's earlier work. Pugh and Quastel have studied amine oxidation in the brain. Although these workers mention mescaline, they have not studied it in detail and consequently have not shown that the properties of its oxidation differ from those of the other amines.

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