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Oxidation of 2-phenylethylhydrazine by monoamine oxidase

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2-PHENYLETHYLHYDRAZINE (also known as Nardil and Phenelzine) is one of the large class of hydrazine derivatives which have been used as antidepressant drugs (see e.g. Refs. 1, 2). These compounds are believed to exert their pharmacological effects primarily by inhibiting monoamine oxidase. Clineschmidt and Horita[3,4] have shown that 2-phenylethylhydrazine can act both as a substrate and an inhibitor of crude preparations of rat liver monoamine oxidase. Although these workers did not identify the immediate product of the enzymic oxidation they concluded that the mechanism probably involved dehydrazination.

Pig brain monoamine oxidase was prepared by the method previously reported.[5] 2-phenylethylhydrazine-1[^{14}C]hydrochloride was prepared by the reaction of 2-phenylethylamine-1^{14}C with hydroxylamine-O-sulphonic acid.[6] The labelled amine was obtained from New England Nuclear Corporation, Boston, Mass. and hydroxylamine-O-sulphonic acid was prepared by the method of Matsuguma and Andrieth.[7] Phenylacetaldehyde was redistilled in an atmosphere of nitrogen before use. Phenylethylidenehydrazine was prepared by the reaction of phenylacetaldehyde with an excess of hydrazine (see e.g. Ref. 8) and phenylacetaldehydephenylethylhydrazone was prepared in a similar way. Ammonia was determined colorimetrically with Nestler's solution after diffusion in Conway dishes.[9] Spectra were determined in a Cary 15 spectrophotometer. Thin layer chromatography was carried out on silica-gel plates developing with benzene–ethylacetate (80 : 20, v/v). The marker compounds were applied in methanolic solution and they were stained by exposure to iodine vapour. The positions of the radioactive spots were determined with a Panax T.L.C. scanner.

When monoamine oxidase was incubated at 25° with 1·0 mM 2-phenylethylhydrazine in 0·2 M phosphate buffer (pH 7·2) for periods of up to 90 min no liberation of ammonia could be detected. Thus an oxidation mechanism in which the terminal amino group was removed to produce phenylethylamine, which itself could be further oxidized to the aldehyde can be ruled out. Since the oxidation of amines by monoamine oxidase is believed to involve the formation of an imine as an intermediate,[10]

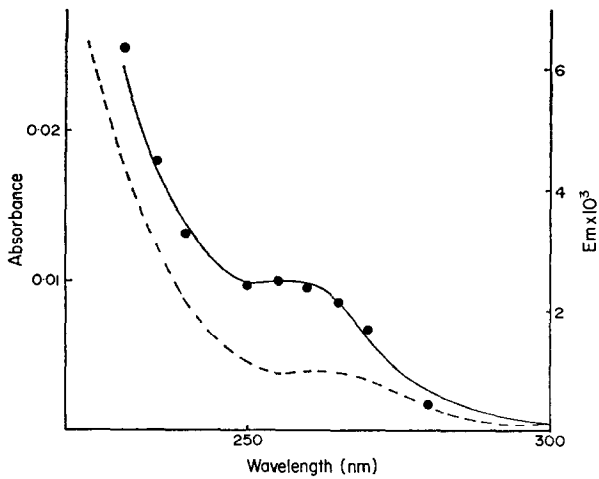

FIG. 1. Spectrum of the product of 2-phenylethylhydrazine oxidation. Monoamine oxidase was incubated for 60 min at 30°, in 0·2 M phosphate buffer (pH 7·2), with 50 μM 2-phenylethylhydrazine. The spectrum of the mixture was then determined against a reference cuvette containing the buffer plus enzyme. The results are shown by the solid line. The absorbance of 2-phenylethylhydrazine at the concentrations used is negligible above 220 nm. The initial rates of increase in absorbance (in arbitrary units) at a series of wavelengths when monoamine oxidase was incubated with 250 μM 2-phenylethylhydrazine under conditions similar to those above.

The broken line represents the absorption spectrum of phenylethylidenehydrazine

the oxidation of a hydrazine could, by analogy, involve a dehydrogenation at the carbon–nitrogen bond to form a hydrazone. Such a mechanism would be consistent with the fact that monoamine oxidase is active towards *N*-substituted amines.[11]

The ultra-violet absorption spectrum of the product of enzymic oxidation of 2-phenylethylhydrazine is shown in Fig. 1. This spectrum is not consistent with either phenylacetaldehyde or phenylacetic acid being the major product of the reaction.[12] The spectrum is, however, similar to that of phenylethylidenehydrazine, the hydrazone that would be formed by dehydrogenation of 2-phenylethylhydrazine at the carbon–nitrogen bond. The spectrum of this compound is also shown in Fig. 1.

It is possible that the spectrum determined in this way does not represent that of the immediate product of the oxidation reaction. The immediate product could have reacted further to produce the absorption spectrum observed. If phenylacetaldehyde were the immediate product of the reaction this could react further with the excess 2-phenylethylhydrazine present to form the corresponding substituted hydrazone: phenylacetaldehydephenylethylhydrazone. In order to investigate this possibility the initial rates of change in absorbance were determined at a series of wavelengths when monoamine oxidase was incubated with 2-phenylethylhydrazine. The results of these determinations are shown in Fig. 1 and the agreement between the absorption spectra obtained by these two methods indicates that, if any further reaction of the immediate product takes place it must be extremely rapid. Although phenylacetaldehydephenylethylhydrazone has a similar absorption spectrum to phenylethylidenehydrazine its formation from phenylacetaldehyde and 2-phenylethylhydrazine was found to occur relatively slowly under these conditions.[13]

When monoamine oxidase was incubated with 1 mM [^{14}C]labelled 2-phenylethylhydrazine in 0·2 M phosphate buffer (pH 7·2) for 60 min and the mixture was subjected to thin layer chromatography two radioactive spots were separated, that corresponding to unchanged 2-phenylethylhydrazine which remained at the origin and a second spot (R_f value 0·6) which ran with a phenylethylidenehydrazine marker. No spots corresponding in position to phenylacetaldehyde, phenylacetic acid or phenylacetaldehydephenylethylhydrazone could be detected. Thus it appears that the product of 2-phenylethylhydrazine oxidation by monoamine oxidase is the corresponding hydrazone.

The reaction pathway of pig brain monoamine oxidase has been shown to proceed by way of a free reduced form of the enzyme[14] and the possible pathways of hydrazine oxidation and amine oxidation are compared in Scheme 1. The formation of the hydrazone as a product of hydrazine oxidation provides evidence in support of the theory that amine oxidation proceeds by way of an imine.[10]

$$E + RCH_2NH_2 \rightarrow EH_2 + RCH{:}NH$$
$$RCH{:}NH + H_2O \rightarrow RCHO + NH_3$$
$$EH_2 + O_2 \rightarrow E + H_2O_2$$

$$E + RCH_2NHNH_2 \rightarrow EH_2 + RCH{:}NNH_2$$
$$EH_2 + O_2 \rightarrow E + H_2O_2$$

SCHEME 1. The pathway of amine and hydrazine oxidation by monoamine oxidase.
Upper: amine oxidation. Lower: hydrazine oxidation.

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REFERENCES

1. J. H. BIEL, A. HORITA and A. E. DRUKKER, in *Psychopharmacological Agents* (Ed. M. GORDON) p. 359. Academic Press, New York (1964).
2. M. CHESSIN, B. DUBNICK, G. LEESON and C. C. SCOTT, *Ann. N.Y. acad. Sci.* **80,** 597 (1959).
3. B. V. CLINESCHMIDT and A. HORITA, *Biochem. Pharmac.* **18,** 1011 (1969).
4. B. V. CLINESCHMIDT and A. HORITA, *Biochem. Pharmac.* **18,** 1021 (1969).
5. K. F. TIPTON, *Europ. J. Biochem.* **4,** 103 (1968).
6. R. GOSL and A. MEUWSEN, *Org. Syntheses* **43,** 1 (1963).

7. H. J. MATSUGUMA and L. AUDRIETH, *Inorg. Syntheses* **5**, 122 (1957).
8. D. E. PEARSON, K. N. CARTER and C. M. GREER, *J. Am. chem. Soc.* **75**, 5905 (1953).
9. E. J. CONWAY, *Biochem. J.* **27**, 419 (1933).
10. M. L. C. BERNHEIM, *J. biol. Chem.* **93**, 299 (1931).
11. H. BLASCHKO, *J. Physiol., Lond.* **153**, 17 (1960).
12. E. C. C. BALY and F. G. TRYHORN, *J. chem. Soc.* **107**, 1058 (1915).
13. K. F. TIPTON, in *Mechanisms of Toxicity* (Ed. W. N. ALDRIDGE), p. 13. Macmillan, London (1971).
14. K. F. TIPTON, *Europ. J. Biochem.* **5**, 316 (1968).

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Effect of 3,5-diethoxycarbonyl-1,4-dihydro collidine on the metabolism of iron in mouse liver

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MANY chemically unrelated drugs stimulate the hepatic formation of porphyrins in the experimental animals and in liver cells cultured *in vitro*.[1,2] These drugs enhance the activity of the hepatic 5-amino-laevulinate (ALA) synthetase,[3] the rate limiting enzyme in the biosynthetic pathway of porphyrin and haem, but the exact mechanism underlying this effect is not yet known. Granick[3] has suggested that the chemicals that induce porphyria act by interfering with the feed-back control exercised by haem at the level of ALA synthetase.

There are three main mechanisms by which the porphyrinogenic drugs may interfere with the feed-back control exercised by haem: (1) the drugs might inhibit the synthesis of haem; (2) they might compete with haem for some regulatory site where haem has to become bound in order to control ALA synthetase or (3) they might increase the rate of haem utilization or breakdown.

Evidence has been presented in favour of each of these three mechanisms.[3–5] For example, Onisawa and Labbe have described an inhibition of haem synthesis in mice made porphyric by 3,5-diethoxy-carbonyl-1,4-dihydro collidine (DDC).[4] Since their experiments were carried out on animals treated for several days with relatively high doses of DDC, a decreased iron-protoporphyrin chelation due to an aspecific toxic effect of the drug cannot be excluded. Moreover in a recent study we found that 5-ALA synthetase activity reached a maximum within a few hours after DDC administration[6] whereas the inhibition of the ferrochelatase had been observed by Onisawa and Labbe after 7–10 days of treatment.[4]

For these reasons a further study of the effect of DDC on the incorporation of ^{59}Fe by the liver has now been studied *in vivo* and *in vitro* within a few hours of DDC administration.

Male Swiss S.M. mice weighing 30 ± 4 (S.D.) g were used. The DDC was prepared according to the method of Eisner[7] and dissolved in corn oil at the concentration of 20 mg/ml. Ferric chloride (^{59}Fe) solution (0·2 mc/ml) was supplied by Sorin (Saluggia, Italy). Animals were fasted overnight before dosing and their fasting continued until they were killed. They were injected intraperitoneally with DDC solution (133 mg/kg body weight) and killed 4 hr later; control mice received the same volume of corn oil alone. The *in vivo* uptake of ^{59}Fe into liver tissue and its incorporation into liver haem were determined at several intervals varying from 15 to 120 min after i.p. injection of 3 μc of ^{59}Fe/mouse. The incorporation of ^{59}Fe into liver haem was also studied *in vitro* using both liver homogenates[8] and isolated mitochondria.[9] Liver haem was crystallized by the method of Labbe and Nishida,[10] using red blood cells to supply carrier haem. The specific activity of the crystallized haem[11] and the radioactivity of total liver homogenate were determined in a well type scintillation counter. All determinations were done in duplicate.

The effect of a single i.p. injection of DDC on the liver uptake of ^{59}Fe and on its incorporation into haem is shown in Table 1.