The mechanism of oxidation of amphetamine enantiomorphs by liver microsomal preparations from different species

A recent communication (Parli, Wang & McMahon, 1971) on the mechanisms of oxidation of (+)-amphetamine by rabbit liver oxygenase proposes that the initial step leading to the production of the two major metabolites, phenylacetone oxime and phenylacetone, involves hydroxylation at the C-atom in the α-position to the amino-group. We have found the metabolism of (+)- and (−)-amphetamine by microsomal preparations of rabbit, guinea-pig and rat to implicate oxidation on the nitrogen to give α-methyl β-phenethylhydroxylamine as the metabolic route which then leads to *syn* and *anti*-phenylacetone oximes.

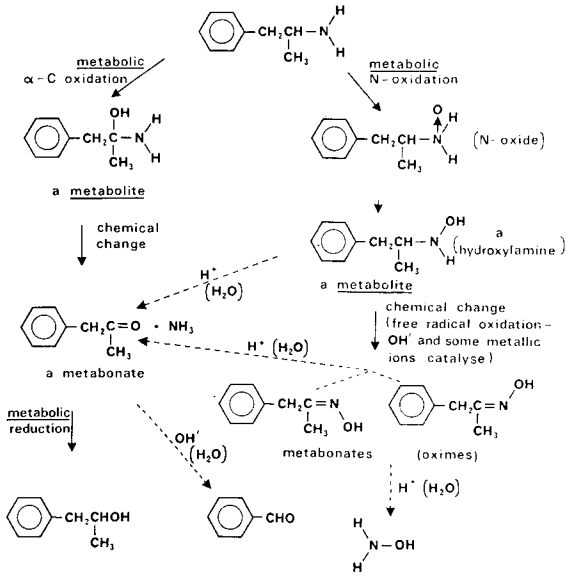
The presence of α-methyl-β-phenethylhydroxylamine in the solution after incubation of the amphetamine substrate with the microsomal preparation (10 000 *g* supernatants as well as microsomes) was demonstrated by analysis of organic extracts by: g.l.c.: retention time 8·5 min on 2m glass column, 7·5% Carbowax 20 M on Chromosorb W 80–100 mesh, N_2,30; H_2,20; Air 20 p.s.i.; t.l.c.: R_F 0·63 on silica gel, solvent methanol–chloroform (1 : 4); extraction of this spot and injection onto g.l.c. gave the peak stated above; *polarography:* $E_{\frac{1}{2}}$, −0·50; 2 μg/ml in Na_2SO_3/NaOH solution (pH 12·4) at 20°; *chemical change* to *anti-* and *syn*-phenylacetone ketoximes (2 : 1) by shaking the solution with air in aqueous alkaline solution.

In all the above tests, identical results were obtained using synthetic α-methyl-β-phenethylhydroxylamine. This compound can be extracted from neutral aqueous solution with ether or benzene and these extracts can be evaporated to low bulk without decomposition, but extraction by ethyl acetate and evaporation leads to extensive decomposition into phenylacetone and its oximes as well as other products. The hydroxylamine was not metabolized by microsomal enzymes.

Unlike the results quoted by Parli & others (1971), we found only small amounts of phenylacetone oxime (*anti-* and *syn*-forms) to be present after incubation of amphetamine; these were extracted with ether and examined by g.l.c. and t.l.c. No traces of phenylacetone imine could be detected.

Since we have shown previously that the respective routes yielding metabolites giving the ketone or oxime respectively as metabonates may be blocked selectively by inhibitors (Beckett, Van Dyk & others, 1971), the metabonate, phenylacetone, in the present work must arise primarily from the metabolite resulting from α-C-oxidation. However, additional phenylacetone can be produced chemically from α-methyl-β-phenethylhydroxylamine or phenylacetone oximes if storage, extraction and analytical techniques are inappropriate.

Parli & others (1971) proposed that phenylacetone oxime arises as a result of α-C-oxidation followed by dehydration to phenylacetone imine and then metabolic *N*-oxidation. However, in aqueous solution, the derived α-carbinol amine would be expected to undergo conversion to phenylacetone and ammonia in preference to elimination of water to give the imine. Even when elimination from the α-carbinol amine would lead to a conjugated imine as in a benzyl attached to the amine group (e.g. benzylamphetamine as substrate) we found only traces of the imine (Beckett, 1972). Even if the imine from amphetamine is postulated as being metabolized to phenylacetone oxime, it seems very unlikely that the microsomal enzymes would hydrate the imine metabolically to give the hydroxylamine which in our experiments is present after incubation when there are only small amounts of the oximes. Also, *N*-hydroxy compounds have been obtained after microsomal attack on "phentermines" in which there is no hydrogen on the α-carbon to allow formation of an α-carbinolamine or imine (Beckett & others, unpublished).



Formation of metabolites and metabonates from AMPHETAMINE by N- and α-C metabolic oxidation.

We therefore conclude that microsomal enzymatic attack on amphetamine about the *N*-centre occurs by two routes: (i) on the carbon at the α-position to the *N*-atom to give the α-carbinol amine and (ii) on the *N*-atom to give the *N*-oxide which by proton transfer becomes the hydroxylamine (see above). The α-carbinol amine breaks down in neutral acidic or alkaline aqueous solution to phenylacetone and ammonia. The hydroxylamine in neutral incubation liquid can be extracted into ether unchanged, but in acidic solution and in some organic solvents, it breaks down into the phenylacetone oximes and phenylacetone, and in alkaline solution breaks down into the oximes. The possibility under certain conditions of some metabolic dehydrogenation of metabolically produced α-methyl-β-phenethylhydroxylamine to yield phenylacetone oximes is not excluded. The oximes are stable in alkaline solution but are changed quickly in acid solution and more slowly in neutral solution to phenylacetone.

The explanation is consistent with the 95% incorporation of ^{18}O into phenylacetone oxime observed by Parli & others since the oxygen in the oxime is derived by free radical abstraction of hydrogen from the metabolically produced α-methyl-β-phenethylhydroxylamine. The low incorporation (30%) of ^{18}O into phenylacetone reported by Parli & others could be explained by most of the phenylacetone being produced by the chemical breakdown of the α-methyl-β-phenethylhydroxylamine metabolite or its oxime metabonates in the procedures used by these authors before the g.l.c. mass spectral analysis.

Using 10 000 *g* microsomal preparations, the relative importance of the metabolic *N*- and the α-C-oxidation routes for (+)- and (−)-amphetamine in various species as indicated by the quantities of α-methyl-β-phenethylhydroxylamine (+ a small amount of the oximes) and phenylacetone (+ its alcohol) found was:– rabbits: the *N*-route was equivalent to the α-C-route and (−)-amphetamine was about 8 times better as a substrate than the (+)-isomer; guinea-pigs: the *N*-route was 10 times greater than the α-C-route and (−)-isomer was about 0·5 times as good a substrate as the (+)-isomer; rats showed only about 1/100 activity of guinea-pigs in the *N*-oxidation route, but the α-C-route was negligible and the isomers were equivalent as substrates.

We thank the Medical Research Council for financial assistance, and one of us (S. Al-Sarraj) thanks the University of Baghdad for sabbatical leave.

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December 16, 1971

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A simple method for the preparation of injectables of tetrahydrocannabinols and cannabis extracts

Because of the increased use of tetrahydrocannabinols and cannabis extracts in the experimental laboratory, an inexpensive method that requires minimum effort and equipment is needed to produce a reliable aqueous suspension of these highly water-insoluble drugs. Such a method was developed in our laboratory while studying the electro-encephalographic effects of synthetic 1-*trans*-Δ^9-tetrahydrocannabinol (Δ^9-THC), 1-*trans*-Δ^8-tetrahydrocannabinol (Δ^8-THC) and marihuana extract distillate (MED) (Moreton, unpublished doctoral dissertation).

Resinous Δ^9-THC and Δ^8-THC (95% pure) and liquid MED (17·1% Δ^9-THC) were supplied by the National Institute of Mental Health. They were kept frozen at −20° before preparation of injections.

Frozen Δ^9-THC or Δ^8-THC or liquid MED was mixed in a glass mortar and pestle with a quantity of Arlacel-20 equal to 2% (w/v) of the intended final volume until all material was dissolved. An equal amount of Tween-65 was then added and thoroughly mixed. One ml of physiological saline was added and mixed to form a creamy suspension. This was repeated several times and then the suspension made up to produce suspensions containing 20 mg/ml of the drug.

The drugs were rapidly released from the suspension as indicated by behavioural and eeg measurements, while the control vehicle was found to be inactive.

In contrast to the polyvinylpyrrolidone suspension described by Fenimore & Loy (1971), the Arlacel-Tween suspension is especially suitable when concentrations greater than a few mg per ml are desired.

The surfactants were obtained from the Surfactant Supply Division, Emulsion Engineering Inc., Elk Grove Village, Illinois.

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November 24, 1971

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