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The Reaction of Monoamine Oxidase with Phenethylhydrazine

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The enzyme monoamine oxidase [monoamine-oxygen oxidoreductase (deaminating), EC 1.4.3.4] has been shown to catalyse the oxidative deamination of a wide variety of amines, producing the corresponding aldehyde, ammonia and hydrogen peroxide (Blaschko, Richter & Schlossmann, 1937). The kinetic reaction mechanism has been studied with purified preparations of the enzyme from pig brain mitochondria (Tipton, 1968*a*) and from ox liver mitochondria (Oi, Shimada, Inamasu & Yasunobu, 1970). In both cases it has been shown that the kinetic reaction pathway is one in which there is no kinetically significant formation of a ternary complex of the enzyme with oxygen and the amine, the reaction taking place by way of a series of binary complexes with a free modified form of the enzyme being produced as an intermediate.

The modified form of the enzyme that is implicated by the kinetic results probably represents a reduced or partially reduced form, and the enzymes prepared from ox liver (Gomes, Igaue, Kloepper & Yasunobu 1969), ox kidney (Erwin & Hellerman, 1967), pig brain (Tipton, 1968*b*) and rat liver (Sourkes, 1968) have all been shown to contain FAD bound to the enzyme in a ratio of approx. 1 mol of flavin/100 000 g of enzyme protein. In the presence of an amine substrate this flavin has been shown to become reduced. By analogy with the amino acid oxidases (Frieden & Velick, 1957), it may be that the immediate product of amine oxidation is an imine, which then decomposes in the presence of water to form the aldehyde plus ammonia.

A wide variety of hydrazine derivatives have been found to be inhibitors of monoamine oxidase and these compounds have found clinical use as anti-

depressants (for review see Biel, Horita & Drukker, 1964). One of the simplest of these compounds is phenethylhydrazine (also known as phenelzine and Nardil). This compound has been shown to be a powerful monoamine oxidase inhibitor both *in vivo* and *in vitro* (see, e.g., Chessin, Dubnick, Leeson & Scott, 1959) and it has found wide therapeutic use.

Recently Clineschmidt & Horita (1969*a,b*) have shown that, as well as acting as an inhibitor of monoamine oxidase, phenethylhydrazine also acts as a substrate for the enzyme. These authors did not identify the product of the reaction, which they suggested was phenylacetaldehyde.

Studies on the u.v.-absorption spectrum of the products of the reaction have shown that the spectral properties are not consistent with phenylacetaldehyde being a major reaction product, the spectrum being more consistent with the formation of a hydrazone. If phenylacetaldehyde were formed as a product of the reaction it could rapidly react with the excess of phenethylhydrazine present to form the corresponding phenethylhydrazone. This compound has been synthesized and shown to be a relatively weak reversible inhibitor of monoamine oxidase (Tipton, 1970).

Alternatively the major product of the reaction could be phenylacetaldehyde hydrazone, this compound being formed in the same way as the imine derived from normal amine oxidation, but not being as readily hydrolysed to the corresponding aldehyde. Support for this theory comes from the failure to detect the formation of ammonia during the course of phenethylhydrazine oxidation by monoamine oxidase. The hydrazone generated on the enzyme surface may represent the actual inhibitory species formed from phenethylhydrazine.

Support for the view that oxidation of phenethylhydrazine is intimately linked with monoamine oxidase inhibition comes from studies on the kinetics of the inhibition process. Inhibition by phenethylhydrazine has been shown to be dependent on the presence of oxygen, as was previously reported for the inhibition of monoamine oxidase by *N*-isonicotinoyl-*N'*-isopropylhydrazine (iproniazid) by Davison (1957). The variation of the initial rate of activity loss with oxygen concentration can be interpreted as a Michaelis curve, and the K_m calculated from this is in close agreement with the K_m for oxygen calculated for the direct oxidation of phenethylhydrazine catalysed by monoamine oxidase. Similarly the K_m value calculated from studies of the effect of phenethylhydrazine concentration on the initial rate of inhibition is similar to the K_m value for the oxidation of this compound. The variation of the initial rate of monoamine oxidase inhibition with pH has been found to closely parallel the variation of the initial rate of phenethylhydrazine oxidation with pH. The

presence of substrate protects monoamine oxidase from inhibition by phenethylhydrazine, and the kinetics of benzylamine oxidation in the presence of the inhibitor can be described by a kinetic mechanism involving two competitive reactions.

These results suggest that the oxidation of phenethylhydrazine by monoamine oxidase and the inhibition of the enzyme may be interdependent phenomena.

Studies on the kinetic reaction pathway of phenethylhydrazine oxidation indicate that this monoamine oxidase-catalysed reaction may follow a different kinetic mechanism from the oxidation of more normal substrates. The kinetics are consistent with a mechanism in which ternary complexes are formed and in which no kinetically significant quantities of the free modified enzyme are formed. A kinetic reaction pathway in which the first product does not readily dissociate from the enzyme before the second substrate (oxygen) is bound will fit the data. This would be in accord with the formation of an enzyme-bound hydrazone, which, being resistant to hydrolysis, would not easily dissociate from the enzyme.

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Monoamine Oxidase: Multiple Forms and Selective Inhibitors

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Mitochondrial monoamine oxidase [monoamine-oxygen oxidoreductase (deaminating), EC 1.4.3.4] from various human, rat and bovine organs has been separated by electrophoresis into constituent isoenzymes (Youdim & Sandler, 1967; Kim & D'Iorio, 1968; Collins, Youdim & Sandler, 1968; Youdim, Collins & Sandler, 1969; Gomes, Igaue, Klopfer & Yasunobu, 1969; Gorkin, 1969; Sierens & D'Iorio, 1970). Purified enzyme from human and rat liver mitochondria, for example, exists in five enzymically active forms (Youdim, Collins & Sandler, 1970) whereas that from the brain of both species separates electrophoretically into four active bands (Youdim *et al.* 1969; Collins, Sandler, Williams & Youdim, 1970). The isoenzymes exhibit different physicochemical properties: thermal stability, pH optimum and substrate and inhibitor specificity all vary, sometimes in different regions of the same organ.

Monoamine oxidase inhibitors have been widely used in the treatment of depressive illness, and it has been tacitly accepted that their beneficial effect stems from inhibition of monoamine oxidase alone and the resulting elevation of monoamine concentrations in the central nervous system (Pletscher, Gey & Burkard, 1966). There had previously been no convincing explanation as to why some drugs inhibiting the enzyme are effective in the treatment of depression and others are not (Davis, 1965; Hendley & Snyder, 1968). It would now seem reasonable to suggest that the answer lies in terms of differential inhibition of monoamine oxidase isoenzymes. This hypothesis brings in its train an important corollary: if it were to prove to be correct, it follows that the synthesis of specific inhibitors tailored to an individual isoenzyme band at a particular anatomical site should be within our grasp. Implicit in this argument is the conclusion that the therapeutic effectiveness of monoamine oxidase inhibitors relies on a localized accumulation of a particular amine substrate at a specific site in the brain. The pinpointing of this site is one of the eventual aims of our study. Such is the strategy: this communication outlines some of the tactics.

Most of our efforts in the complex field of inhibitor studies have been concentrated on two organs, liver and brain. The inhibitory effect of iproniazid, pheniprazine, pargyline, harmaline and clorgyline (M&B 9302) *in vitro* on four of the five forms of rat liver monoamine oxidase was measured with four