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COMMUNICATIONS

On the stereospecificity of monoamine oxidase

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The recent report by Fowler & Oreland (1981) on the stereoselective inhibition of monoamine oxidase (MAO) by 4-dimethylamino-α-2-dimethyl phenylethylamine refers to some interesting points concerning the stereospecificity of the enzyme which may benefit from further discussion. It is true that hydrogen abstraction from the α-carbon atom of tyramine by MAO is stereospecific (Belleau et al 1960) and that α-methyl substituted phenylalkylamines such as amphetamine or α-methyl benzylamines are stereoselective inhibitors. However, it is important to stress that stereospecificity or stereoselectivity shown by the enzyme at the α-carbon atom of substrates or inhibitors should not be confused with that shown towards amines in which the chiral centre is at the β-carbon. The topographic features of MAO which confer such stereoselectivity almost certainly differ for those two positions. This is most clearly demonstrated by the fact that α-substitution, as in amphetamine, does not markedly influence binding of amines to the enzyme because this compound is an excellent competitive inhibitor, but it does affect catalysis, as amphetamine has long been regarded as a non-substrate. On the other hand, amines such as noradrenaline which have a chiral centre at the β-position both bind to, and are oxidized by, MAO. Since both the optical isomers of noradrenaline are oxidized by MAO it seems unlikely that proton abstraction, stereoselective or otherwise, from the α-carbon is determined by a particular configuration at a chiral β-position, though it should be stressed that stereospecific removal of a pro-*R*-α-hydrogen by MAO may not be universally applicable, since it has been demonstrated only for two substrates, n-heptylamine (Battersby et al as reported by Staunton & Summers 1978) and tyramine, neither of which has a chiral centre at the β-position. The oxidation of both isomers of noradrenaline by MAO could be explained in stereochemical terms if the A and B forms of the enzyme had opposite specificities, so that in an A/B mixture both isomers would be metabolized. This seems an unlikely explanation because in the case of β-hydroxy-β-phenylethylamine, for example, the (+)-isomer appears to be a substrate for both forms of MAO (Williams 1977).

The situation is further complicated by a number of other factors. Firstly, the stereoselectivity shown towards (+)-amphetamine by MAO is the opposite of that seen with the irreversible inhibitors *N*-propargyl amphetamine (Williams & Dollie 1978) and its *N*-methyl-analogue selegiline (deprenyl) (Knoll & Magyar 1972). For both these amphetamine derivatives the (−)-isomers are the more potent inhibitors. (It is assumed that the sign of rotation and absolute configuration are consistent in amphetamine and its derivatives. This seems to be the case for *N*-propargyl amphetamine (Williams & Dollie 1978). Secondly, amphetamine shows a preference for MAO-A, whereas the two propargyl derivatives are both B-selective. Again these differences cannot be ascribed to opposite stereospecificities of the A and B forms of MAO because (+)-amphetamine inhibits both forms of the enzyme more effectively than its (−)-isomer. The examples of 2,3-dichloro-α-methyl benzylamine (Fuller & Hemrick 1978) and the inhibitor described by Fowler & Oreland (1981) where selectivity and chirality *are* associated in this way are thus extremely interesting. Further investigations of phenomena such as these may help to clarify what is at present a complex situation. However, the compound described by Fuller & Hemrick (1978) raises a quite different point concerning chirality and specificity. It might appear that its (+)-isomer, in being selective for MAO-A, parallels (+)-amphetamine. This may not be so, because although these two compounds have the same sign of rotation, (+)-α-methyl benzylamine has an *R*-configuration (Bush et al 1969) the opposite of *S*-(+)-amphetamine, suggesting that its 2,3-dichloro derivative may be *R* also. This emphasizes the importance of taking account of absolute configurations of optically active compounds when considering biological specificity.

It is clear that introduction of an α-substituent into substrates or inhibitors of MAO can significantly affect A/B selectivity, though not necessarily in a predictable way. The non-selective *N*-propargyl-β-phenylethylamine (Williams & Lawson 1974) gives rise to the B-selective *N*-propargyl amphetamine and introduction of $CHCH_3$ into the side chain of pargyline, giving rise to selegiline, produces a marked shift in favour of MAO-B. On the other hand α-methylation of the MAO-B substrate, β-phenylethylamine produces the A-selective amphetamine. The author has found that (±)-α-methyl clorgyline discriminates in favour of MAO-A as effectively as clorgyline (Williams, unpublished observation).

It is obvious from much of the foregoing that chain

branching rather than chirality itself is important in producing selectivity. Hence the compounds described by Fuller & Hemrick (1978) and Fowler & Oreland (1981) can be seen as special examples of this general case such that introduction of a chiral centre has produced selective inhibitors in which other structural features have fortuitously conferred the observed optical specificity. Study of such compounds may lead to the production of better selective inhibitors of the A and B forms of MAO.

Belleau et al (1960), using *R* and *S* isomers of α-deutero tyramine as substrates for MAO suggested from observed kinetic isotope effects that the α-carbon is a pro-chiral centre from which the enzyme stereospecifically removes the pro-*R*-hydrogen. This hydrogen atom has the same orientation in space as the α-hydrogen of *S*-(+)-amphetamine when the amino groups and aromatic rings of these two amines are superimposed. This suggests that (+)-amphetamine may be a better fit at the active site of MAO than its enantiomer, which would be consistent with the finding that (+)-amphetamine is the better competitive inhibitor.

In a comparable conformation, it is the α-methyl group in (−)-amphetamine which occupies this position. How then can the optical preference of (−)-selegiline and (−)-*N*-propargyl amphetamine be reconciled with that of (+)-amphetamine? It is known that propargylamines first bind reversibly at the active site of MAO, followed by a time-dependent phase (Tipton & Mantle 1981) in which a covalent bond is formed between enzyme and substrate initiated by abstraction of a proton, not from the usual α-carbon, but from the methylene carbon of the propargyl group. By analogy with (+)-amphetamine it may be postulated that (+)-selegiline and (+)-*N*-propargyl amphetamine are a better fit than their enantiomers at the active site. If so, they would hold the enzyme in a conformation in which the α-hydrogen was bound to its appropriate site, thus reducing the possibility of proton abstraction from the propargyl group. Conversely, the (−)-isomers are likely to be a poorer fit and therefore less likely to impede, indeed may facilitate, the conformational change in the enzyme necessary to bring the appropriate hydrogen in the propargyl group into juxtaposition with the site of proton transfer.

Recently, Yu et al (1982), extending the work of Belleau and his co-workers, have confirmed that αα-dideuterated amines (*p*-tyramine, *m*-tyramine, β-phenylethylamine) show kinetic isotope effects (increase in K_m and fall in V_{max}) though the values obtained for αα-d_2-tyramine differ from those reported earlier. The corresponding ββ-d_2-amines differed little from the hydrogen-containing analogues. These findings support the suggestion that α-hydrogen removal from the substrate is involved in the rate-determining step. The isotope effects shown by αα-d_2-phenylethylamine were much less than for *p*-tyramine, perhaps reflecting differences in the A and B forms of MAO. Monodeuterated amines were not examined, so that the only information available on the stereospecificity of the MAO reaction are those already referred to. Further studies of this kind, using the separate A and B enzymes, seem to be called for.

Some asymmetric MAO inhibitors have either never been optically resolved, or their enantiomers have not been separately tested against the enzyme. These include phenyl alkylhydrazines of diverse structures (Biel et al 1959), various compounds loosely based upon selegiline (Knoll et al 1978; Kalir et al 1981) and some reversible inhibitors of the α-methyl phenylalkylamine type (Green & El Hait 1980; Inoue et al 1976). Some are known to be selective inhibitors of MAO and their optical resolution may yield enantiomers of enhanced selectivity. The four stereoisomers of ephedrine are other obvious examples which may repay examination.

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