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SPECIFICITY OF AMINE OXIDASE FOR OPTICALLY ACTIVE SUBSTRATES AND INHIBITORS

BY

P. PRATESI* AND H. BLASCHKO

From the Department of Pharmacology, University of Oxford

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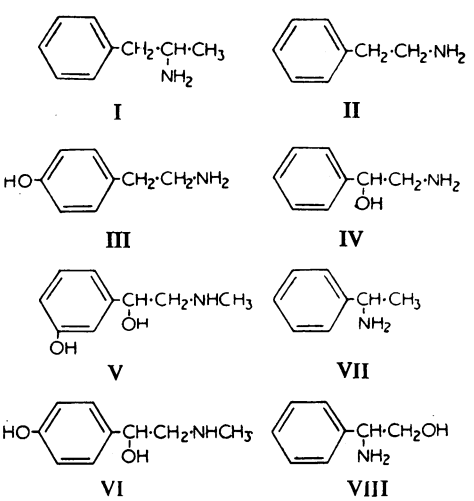
A number of optically active amines have been tested as substrates or inhibitors of amine oxidase of rabbit and guinea-pig liver. The two stereoisomers of β-hydroxyphenethylamine were oxidized at the same rate by rabbit liver, but the guinea-pig liver extracts oxidized the D form more rapidly than the L form. The two stereoisomers of amphetamine were equally active as inhibitors of the rabbit liver oxidase, but with guinea-pig liver extracts dexamphetamine, the (+) form, was more potent as an inhibitor. In both species, 2-hydroxy-1-phenylethylamine was a weaker inhibitor than 1-phenylethylamine; in the rabbit liver (+) forms of these two amines were more potent as inhibitors.

The enzyme amine oxidase, which takes part in the biological inactivation of 5-hydroxytryptamine and many sympathomimetic amines, acts on compounds in which an amino group is attached to a terminal carbon atom. Compounds like amphetamine (α-methylphenethylamine) (I) and its derivatives, substances in which the amino group is not attached to a terminal carbon atom, are not oxidized; they act as competitive inhibitors of the enzyme (see Blaschko, 1952).

Mann and Quastel (1940) suggested that the central stimulating action of amphetamine might be due to its activity as an inhibitor of amine oxidase. This suggestion found some support in the observation that substances like amphetamine, in which the carbon atom adjacent to the benzene ring was not substituted, were stronger central stimulants, and at the same time stronger inhibitors of amine oxidase, than compounds like ephedrine, which carry a hydroxyl group in this position (Blaschko, 1940).

In the present paper the substrate and inhibitor specificities of a number of amines for amine oxidase have been studied. Attention was given to the question whether the steric configuration of amines with an asymmetric carbon atom had an influence upon their affinity for the enzyme. The substrates used were phenethylamine (II), tyramine (*p*-hydroxyphenethylamine) (III) and β-hydroxyphenethylamine (IV); of the last compound the D and L forms were available; the steric configuration of these two forms was determined some time ago (Pratesi and Grassi, 1953). A few experiments with both β*m*- and β*p*-dihydroxy-*N*-methylphenethylamine (V and VI respectively) are also reported. Phenylephrine, the (−) form of compound V, and the (+) form were available; we also used oxedrine (Sympatol), the (±) form of compound VI, as well as the (+) form; the latter compound is now known to have the L configuration (Pratesi, La Manna, Campiglio and Ghislandi, 1958).



The inhibitors tested were: amphetamine (I), 1-phenylethylamine (VII) and 2-hydroxy-1-phenylethylamine (VIII). Of all three compounds the two enantiomorphs were available.

*Present address: Istituto di Chimica Farmaceutica dell'Università di Pavia, Italy.

Material and Methods

The inhibitors used in this study were prepared by Dr. A. La Manna, of the Institute of Pharmaceutical Chemistry at the University of Pavia. All these substances were available as the hydrochlorides. In the following, the prefixes D and L refer to the steric configuration of the compounds; the signs (+) and (−) refer to the optical rotation of the hydrochlorides in aqueous solution.

Most experiments were carried out using a dialysed preparation of freshly ground rabbit or guinea-pig liver. Cat liver has also been used in a few experiments. The liver extracts were prepared in 0.067 M-sodium phosphate buffer of *p*H 7.4, as described by Barlow, Blaschko, Himms, and Trendelenburg (1955). The enzymatic activity was measured manometrically. The manometer flasks contained a fluid volume of 2.0 ml., plus 0.3 ml. of N-KOH. The temperature was 37.5° and the gas phase O_2. Unless stated otherwise, an inhibitor was tipped in from the side bulb, together with the substrate, at the zero time of the experiment. The initial substrate concentration, after tipping, was always 10^{-2} M.

Results

Substrates.—In rabbit liver preparations, the two stereoisomers of β-hydroxyphenethylamine were oxidized at the same rate. In one experiment, the oxygen uptake in the first 20 min. was 32 μl. O_2 for both the D and the L form, whereas with oxedrine, the (±) form of compound VI, the uptake was 18 μl. O_2. Oxedrine was oxidized at the same rate as the (+) form of compound VI.

In the guinea-pig liver extracts, there were two differences: first, oxedrine was always oxidized more rapidly than the two isomers of β-hydroxyphenethylamine, and, secondly, the L form of hydroxyphenethylamine was oxidized more slowly than the D form. In one of these experiments, the oxygen uptakes in the first 20 min. were: with oxedrine 33 μl., with L-β-hydroxyphenethylamine 10 μl. and with D-β-hydroxyphenethylamine 25 μl. There were no differences in the rates of oxidation of *βm-* and *βp*-dihydroxy-*N*-methylphenethylamine by guinea-pig liver extract: oxedrine and its dextrorotatory isomer and phenylephrine and the corresponding laevorotatory form were oxidized at similar rates.

Inhibitors.—In the rabbit liver, the two stereoisomers of amphetamine were equally active as inhibitors. This is illustrated by the following experiments: in one experiment with oxedrine as substrate the inhibitions during the first 15 min. were 91% with both dexamphetamine, the (+) isomer, and (−)-amphetamine in 10^{-2} M concentration; in another experiment the inhibitions were: with 3.33×10^{-3} M dexamphetamine 79%, and with 3.33×10^{-3} M (−)-amphetamine 71%, with 1.67×10^{-3} M dexamphetamine 57% and 1.67×10^{-3} M (−)-amphetamine 66%. These differences are well within the range of experimental error.

The action of the amine oxidase of the rabbit liver on tyramine was less strongly inhibited than that on oxedrine, indicating that tyramine had a higher affinity for the enzyme than oxedrine. In one experiment in which 10^{-2} M dexamphetamine was the inhibitor the inhibition with tyramine as substrate was 78%; with 10^{-2} M (−)-amphetamine as inhibitor it was 80%. In another experiment, the inhibition of the oxidation of tyramine by 10^{-2} M dexamphetamine was 74%; with phenethylamine as substrate the inhibition was 33%. In the rabbit the rate of oxidation of phenethylamine exceeded that of tyramine (see also Alles and Heegaard, 1943); the experiment just described shows that the liver enzyme of this species also has a higher affinity for phenethylamine than for tyramine.

In the guinea-pig liver preparations, tyramine was so much more rapidly oxidized than phenethylamine that it was difficult to compare the oxidation of these two amines in one and the same experiment, using the same amounts of enzyme. Different amounts of the guinea-pig liver extract had therefore to be used with the two substrates. For the oxidation of tyramine, 0.4 ml. of extract was chosen as a suitable amount in each flask; for the oxidation of phenethylamine, 1.6 ml. of the extract was used. Under these conditions, the oxygen uptakes were of a similar order, namely 59 μl. in 15 min. with tyramine and 67 μl. with phenethylamine. The inhibitions in the presence of 10^{-2} M-dexamphetamine were: 95% with tyramine as substrate and 43% with phenethylamine. In other words, although in the guinea-pig liver tyramine was much more rapidly oxidized than phenethylamine (see Blaschko, Richter and Schlossmann, 1937a; Randall, 1946), the enzyme had a higher affinity for phenethylamine than for tyramine.

A comparison of the inhibitory action of the two enantiomorphs of amphetamine revealed a difference between the guinea-pig and the rabbit oxidases. With rabbit liver, there were no marked differences in the inhibitory action of the two isomers, but with guinea-pig liver the dextrorotatory form was always a stronger inhibitor. This difference was noted not only with the two samples of the amphetamine hydrochlorides prepared at Pavia, but it was also present using dexamphetamine (Dexedrine) sulphate and (−)-amphetamine (Levedrine) sulphate, kindly

TABLE I

OXIDATION OF TYRAMINE (10^{-2} M) BY RABBIT AND GUINEA-PIG LIVER PREPARATIONS AND INHIBITION BY THE TWO STEREOISOMERS OF AMPHETAMINE (10^{-2} M)

The values give the oxygen uptakes in μl. during the first 15 min.

	Tyramine Alone	With Dexamphetamine	With (−)-Amphetamine
Rabbit liver ..	66	14·5	13
Guinea-pig liver	71	4·5	17

given by Messrs. Smith, Kline and French. Table I shows the results with two of these experiments, in which 10^{-2} M-tyramine was the substrate.

Amphetamine (I) differs from the other two inhibitors studied, 1-phenylethylamine (VII) and 2-hydroxy-1-phenylethylamine (VIII), in that in amphetamine the amino group is not attached to the carbon atom that carries the phenyl group, whereas in 1-phenylethylamine and 2-hydroxy-1-phenylethylamine both groups are attached to the same carbon atom.

1-Phenylethylamine was found to be a moderately strong inhibitor of amine oxidase. In the rabbit liver extracts (+)-1-phenylethylamine was a more active inhibitor of the enzyme than dexamphetamine. On the other hand, the (+)-2-hydroxy-1-phenylethylamine was a weaker inhibitor than dexamphetamine. Similarly, in two experiments with cat liver preparations, it was found that (+)-1-phenylethylamine was a stronger inhibitor than dexamphetamine.

In the guinea-pig liver extracts dexamphetamine was a stronger inhibitor than either (+)-1-phenylethylamine or (+)-2-hydroxy-1-phenylethylamine. These differences between the rabbit and the guinea-pig liver enzymes are shown in Figs. 1 and 2, taken from experiments in which 10^{-2} M-tyramine served as substrate. It can be seen that the rate of oxidation of tyramine by rabbit liver was most strongly inhibited by 1-phenylethylamine; then comes amphetamine and lastly 2-hydroxy-1-

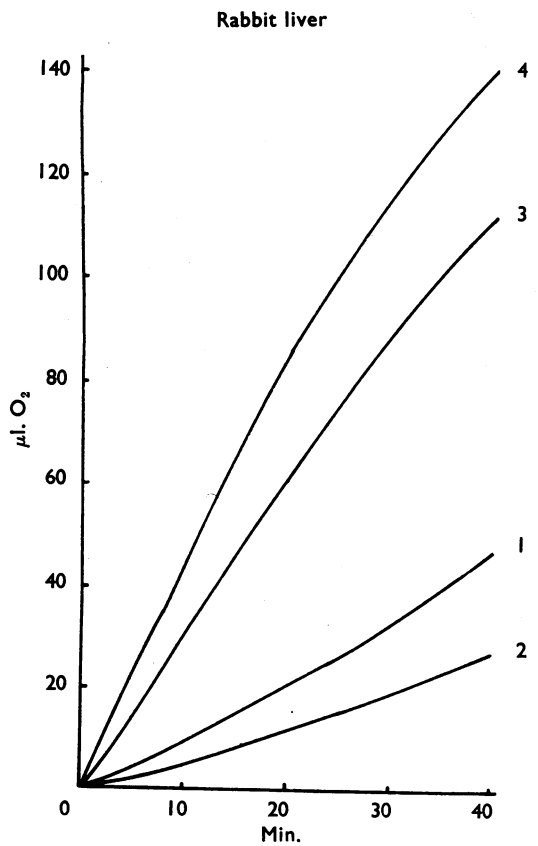


FIG. 1.—Inhibition of amine oxidase of rabbit liver by: (1), dexamphetamine; (2), (+)-1-phenylethylamine; (3), (+)-2-hydroxy-1-phenylethylamine, and (4), tyramine alone. The tyramine concentration and that of the inhibitors was 10^{-2} M.

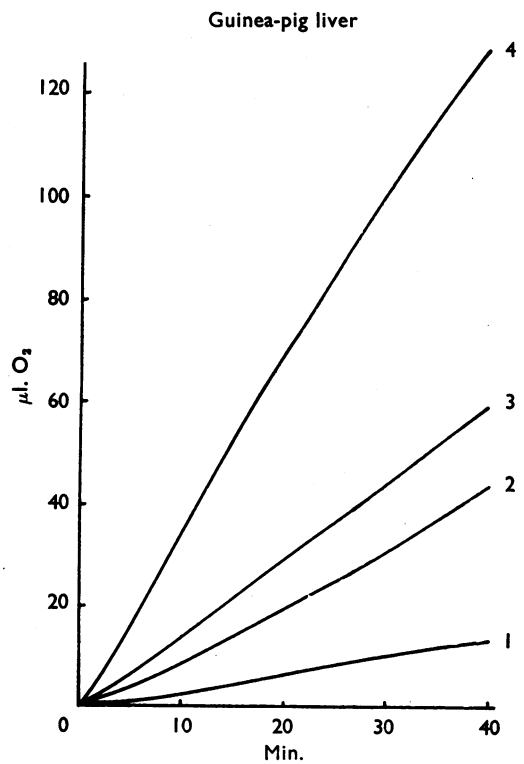


FIG. 2.—Inhibition of amine oxidase of guinea-pig liver by: (1), dexamphetamine; (2), (+)-1-phenylethylamine; (3), (+)-2-hydroxy-1-phenylethylamine, and (4), tyramine alone. The tyramine concentration and that of the inhibitors was 10^{-2} M.

phenylethylamine (Fig. 1); with guinea-pig liver the oxidation was most strongly inhibited by amphetamine (Fig. 2).

The two enantiomorphs of both 1-phenylethylamine and 2-hydroxy-1-phenylethylamine were tested. In the experiments with guinea-pig liver, no difference was noted between the inhibitory activity of the two enantiomorphs, but with rabbit liver the dextrorotatory forms were more active as inhibitors. This was tested in a series of experiments in which 10^{-2} M-oxedrine was used as substrate and an inhibitor concentration of 10^{-3} M. In three such experiments with 1-phenylethylamine the inhibitions in the first 15 min. were: with (−)-1-phenylethylamine, 34, 33 and 12%; and with (+)-1-phenylethylamine, 81, 88 and 62%. In four similar experiments with 2-hydroxy-1-phenylethylamine, the corresponding values were: with (−)-2-hydroxy-1-phenylethylamine, 17, 34, 56, and 52%; and with (+)-2-hydroxy-1-phenylethylamine, 52, 79, 69, and 71%.

Discussion

Many observations have been made on the substrate specificity of amine oxidase, and the observation reported in this paper, that there exist species differences in the pattern of substrate and inhibitor specificities, finds many parallels in earlier work. Little, however, was known of the stereochemistry of the oxidase. In the present work we have been able to test several substances with an asymmetric carbon atom, and of each of these both enantiomorphs were available.

As a result of this study, it can be said that the presence of a centre of asymmetry may confer different affinities (or rates of oxidation) on two enantiomorphs, and also that this difference between two enantiomorphs depends on the source of enzyme. In extracts from rabbit liver, the D and L isomers of β-hydroxyphenethylamine were oxidized at similar rates, but in extracts from guinea-pig liver the D form was more rapidly oxidized than the L form. This observation is of interest in connexion with old observations on adrenaline (Blaschko, Richter, and Schlossmann, 1937b), in which it was found that the initial rate of oxidation of (−)-adrenaline by guinea-pig liver enzyme was faster than that of (+)-adrenaline. That (−)-adrenaline has the D configuration has now been established by a stereochemically unequivocal route (Pratesi, La Manna, Campiglio, and Ghislandi, 1958). Adrenaline, as a catechol derivative, is not a very convenient substrate for manometric experimentation, as oxygen uptake not catalysed by amine oxidase may vitiate results. It is of interest, therefore, that a similar relationship between D and L isomers has now been established for compounds which do not carry a phenolic hydroxyl group.

Another species difference noted was in the relative rates of oxidation of different substrates of the oxidase. In the rabbit liver oxedrine was less readily oxidized than β-hydroxyphenethylamine, but in the guinea-pig liver this order was reversed. The experiments also confirmed the enormous differences in the rate of enzymatic oxidation of phenethylamine, which was rapidly oxidized by the rabbit liver, but slowly by the guinea-pig liver; the experiments with inhibitors suggest that the affinity of the guinea-pig liver enzyme for phenethylamine is high although its rate of oxidation is low.

The well-established general rule, that compounds in which the amino group is not attached to a terminal carbon atom are inhibitors and not substrates of amine oxidase, was confirmed for 1-phenylethylamine and 2-hydroxy-1-phenylethylamine. In the series studied, the introduction of a hydroxyl group in the 2-position relative to the amino group lowers affinity for amine oxidase; this is an analogy in the 1-phenylethylamine series for what is already known in the much more extensively studied group of derivatives of phenethylamine.

A species difference was also found in the comparison of the inhibitory actions of dexamphetamine and (+)-1-phenylethylamine. In the rabbit liver, the latter was the stronger inhibitor (see Fig. 1), but in the guinea-pig liver dexamphetamine inhibited more strongly (see Fig. 2).

A difference was also seen in the inhibitory action of the three inhibitors studied. In the rabbit liver, no significant difference in the inhibitory potency of the two stereoisomers of amphetamine was noted, but both (+)-1-phenylethylamine and (+)-2-hydroxy-1-phenylethylamine were more active as inhibitors than the corresponding laevorotatory forms. Conversely, with guinea-pig liver there was no difference when the two forms of 1-phenylethylamine and 2-hydroxy-1-phenylethylamine were compared, but dexamphetamine was a stronger inhibitor than (−)-amphetamine.

These experiments show that the steric configuration of derivatives of phenylethylamine, of 1-phenylethylamine and of amphetamine may be a factor in determining the affinity for amine oxidase. Can these observations be related to the analeptic properties of the amphetamines? The dextrorotatory form of amphetamine is said to be

more active as an analeptic than the laevorotatory form (see Bovet and Bovet-Nitti, 1948). This is the same difference as that established for the inhibition of the guinea-pig liver enzyme. However, no such difference was found for the rabbit liver enzyme. Moreover, the lack of parallelism between anti-amine-oxidase activity and analeptic action is even more apparent when amphetamine and 1-phenylethylamine are compared. The latter compound was found to be a good inhibitor of amine oxidase, in the rabbit liver even stronger than amphetamine. However, the compound is without the strong central action of amphetamine. This aspect is at present being studied by Drs. E. Grana and L. Lilla at Pavia; they have found that both enantiomorphs of 1-phenylethylamine have a much weaker analeptic action than dexamphetamine in the rat although the action of (+)-1-phenylethylamine on amine oxidase in this species is very similar to that of dexamphetamine. This work will be separately reported. It is difficult to see how these results can be reconciled with the idea that inhibition of amine oxidase can account for the analeptic action of amphetamine and related compounds.

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References

Alles, G. A., and Heegaard, E. V. (1943). *J. biol. Chem.*, **147**, 487.

Barlow, R. B., Blaschko, H., Himms, Jean M., and Trendelenburg, U. (1955). *Brit. J. Pharmacol.*, **10**, 116.

Blaschko, H. (1940). *Nature (Lond.)*, **145**, 26.

—— (1952). *Pharmacol. Rev.*, **4**, 415.

—— Richter, D., and Schlossmann, H. (1937a). *Biochem. J.*, **31**, 2187.

—— —— —— (1937b). *J. Physiol. (Lond.)*, **90**, 1.

Bovet, D., and Bovet-Nitti, F. (1948). *Structure et activité pharmacodynamique des médicaments du système nerveux végétatif*, p. 150. Bâle : Karger.

Mann, P. J. G., and Quastel, J. H. (1940). *Biochem. J.*, **34**, 414.

Pratesi, P., and Grassi, M. (1953). *Farmaco, Ed. sci.*, **8**, 86.

—— La Manna, A., Campiglio, A., and Ghislandi, V. (1958). *J. chem. Soc.*, 2069.

Randall, L. O. (1946). *J. Pharmacol. exp. Ther.*, **88**, 216.