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INHIBITION OF RAT AORTA SEMICARBAZIDE-SENSITIVE AMINE OXIDASE BY 2-PHENYL-3-HALOALLYLAMINES AND RELATED COMPOUNDS

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Abstract—The inhibition of semicarbazide-sensitive amine oxidase (SSAO) in rat aorta homogenates by some 2-phenyl-3-haloallylamines has been studied. Derivatives containing a fluorine atom were approximately three times more potent than the corresponding 3-chloroallylamines. These halogen-containing compounds were irreversible inhibitors of SSAO after preincubation with aorta homogenates; kinetic evidence for an initial competitive, reversible interaction (K_i around 0.4–0.6 μM) was found with two compounds (MDL 72145 and 72274). A similar K_i (approx. 0.7 μM) was obtained with 2-phenylallylamine (MDL 72200). However, this compound which lacks a halogen atom was a reversible inhibitor, even after preincubation. The use of a spectrophotometric assay to measure H_2O_2 production from amine metabolism demonstrated that MDL 72200 was a substrate (K_m = 1.4 μM) for SSAO, with a V_{max} approximately five times smaller than that of benzylamine (K_m = 8.1 μM). Of particular interest in this study is the finding that (E)-2-phenyl-3-chloroallylamine (MDL 72274) is highly selective as an inhibitor of SSAO, compared with MAO-A or B activities, and may be a useful compound for investigating the importance of SSAO in animal tissues.

The mitochondrial enzyme monoamine oxidase (MAO) exists in two forms called MAO-A and MAO-B, which have different substrate specificities and which can be distinguished by their relatively greater sensitivities towards inhibition by the acetylenic compounds clorgyline and deprenyl, respectively. As a consequence, the development of selective inhibitors for these MAO subtypes has attracted considerable interest with the anticipation that this approach may provide therapeutic agents with more advantageous and safer pharmacological profiles compared with non-selective MAO inhibitors in current clinical use (see ref. 1 for review). The selectivities and potential uses of a variety of new compounds, of considerably diverse chemical structure, are currently under evaluation in a number of laboratories. One particular group of drugs acting as irreversible MAO inhibitors is a series of 2-phenyl-3-haloallylamine derivatives [2]. Among these, the MAO-B selective properties of MDL 72145 [(E)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine] have been described in some detail [3, 4].

In addition to MAO-A and B, many animal tissues also contain a semicarbazide-sensitive amine oxidase (SSAO), with particularly high activity in blood vessels where the enzyme is associated predominantly with smooth muscle cells [5, 6]. Although the properties of this enzyme are under continuing investigation, its physiological role and the importance of its inhibition *in vivo* by various drugs remains unknown (reviewed in refs 7 and 8).

We recently reported that MDL 72145 is a potent irreversible inhibitor of SSAO in rat aorta homogenates at concentrations similar to or even below those required to produce MAO-B inhibition in other tissues [9]. In the current study we examined the inhibition of SSAO by related 2-phenyl-3-haloallylamines. From the results obtained it appears that 2-phenyl-3-haloallylamines constitute a new class of inhibitor of SSAO, which may belong to the family of amine oxidases thought to contain copper and pyridoxal phosphate (or another carbonyl reagent-sensitive moiety) as cofactors (see ref. 10). In addition, we have identified one compound [(E)-2-phenyl-3-chloroallylamine, MDL 72274] with high potency and particular selectivity as an inhibitor of SSAO compared with MAO.

Preliminary details of some of these findings have been reported previously [11].

MATERIALS AND METHODS

Animals and chemicals. Adult male Wistar rats (200–500 g) were obtained from our Departmental breeding colony, Animal Services Unit, University of Dundee.

(Methylene-^{14}C)-benzylamine hydrochloride and (G-^{3}H)-5-hydroxytryptamine creatinine sulphate were purchased from Amersham International PLC (Amersham, U.K.). The following drugs and reagents were obtained from Sigma (London) Chemical Co. (Poole, U.K.): 2,2′-azino-bis(3-ethylbenzthiazolinesulphonic acid) (ABTS, diammonium salt), horseradish peroxidase (type II), and the

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hydrochlorides of semicarbazide, hydroxylamine, pargyline and benzylamine. Clorgyline hydrochloride was a generous gift from May & Baker Ltd. (Dagenham, U.K.). The allylamine analogues (hydrochloride salts) were synthesized at the Merrell Dow Research Institute (Strasbourg, France).

Radiochemical assays and preparation of homogenates. Aorta homogenates were prepared in 1 mM potassium phosphate buffer pH 7.8 at a final tissue (g): buffer (ml) ratio of 1:30 for use in the spectrophotometric assay described below, and at 1:320 for use in the radiochemical assay of SSAO with 1 μM [^{14}C]benzylamine as substrate, details of which are given fully in ref. 9.

Solutions of the allylamine derivatives were generally prepared by dissolving hydrochloride salts in 0.2 M potassium phosphate buffer pH 7.8 before use in the radiochemical assay, as previously described for the compound MDL 72145 [9]. However, in the case of the catechol derivatives MDL 72434 and 72486, the buffer also contained 40 μM EDTA (ethylenediamine tetraacetic acid) and 20 μg/ml ascorbic acid as a precaution against possible instability (as may occur with catecholamines such as noradrenaline, etc). Preliminary experiments determined that the final concentrations of these reagents (10 μM and 5 μg/ml, respectively) which were present after inclusion of inhibitor solutions in the assays had no effect themselves on SSAO activity.

MAO assays were carried out with 1:10 liver homogenates, using 100 μM [^{3}H]5-hydroxytryptamine and [^{14}C]benzylamine as respective substrates for MAO-A and B activity.

Dialysis experiments. In order to test whether reversible or irreversible inhibition was produced by drugs, aorta homogenates were preincubated for 20 min with appropriate concentrations of each compound to bring about complete or virtually complete inhibition of SSAO activity. These samples were then dialyzed (as described in ref. 9 for MDL 72145) in an attempt to restore enzyme activity. Dialyzed samples, which also included uninhibited controls, were assayed and compared with corresponding non-dialyzed samples otherwise treated identically. Each experiment was carried out at least twice with different homogenates, each studied in triplicate in a given experiment.

Inhibition kinetics. In an earlier series of experiments to determine the kinetic characteristics of the initial immediate inhibition of SSAO by MDL 72145, we obtained evidence for non-competitive inhibition of benzylamine metabolism during short (1 min) assay incubations [9]. In the latter experiments, assays containing aorta homogenates with various concentrations of substrate and inhibitor were set up in ice-cold tubes before simultaneous transfer of all tubes to a water bath for incubation at 37° for 1 min. At the end of this period, all tubes were ice-cooled rapidly to stop the enzymatic reaction prior to addition of HCl and solvent extraction of metabolites (see ref. 9).

For the current study, this methodology was modified such that the inhibitor and substrate were premixed in assay tubes at 37°, and the enzyme reaction was initiated by addition of homogenate, followed by incubation for 1 min, ice-cooling and immediate addition of HCl. Each assay tube was taken individually through this procedure to provide a strict control on the time of exposure of enzyme to the inhibitor–substrate mixture.

Spectrophotometric assay for SSAO. This method was modified from that of Szutowicz *et al.* [12] for measurement of MAO activity. Assays carried out in triplicate consisted of 0.5 ml 0.1 M potassium phosphate buffer (pH 7.8) containing 3.1 mM sodium azide and appropriate concentrations of amine substrate (benzylamine or MDL 72200). Substrate was omitted in blanks. (Preliminary experiments showed that 3.1 mM sodium azide had no effect upon SSAO activity in rat aorta homogenates when assessed with 1 μM [^{14}C]benzylamine as substrate in the radiochemical assay). Assay tubes were warmed to 37° in a water bath, and the enzyme reaction was started by addition of 25 μl aorta homogenate to each. Incubation with shaking was continued for either 30 min (benzylamine) or 60 min (MDL 72200). At these times, to each tube was added 0.5 ml 0.5 M sodium phosphate–citrate buffer (pH 4.0) containing 1.8 mM ABTS and 5 U horseradish peroxidase. Although in the original published method for MAO [12] sodium dodecyl sulphate (SDS) in HCl was added to stabilize the coloured product (oxidized ABTS) formed, in the presence of the potassium phosphate buffer used in the initial incubation in our system, SDS was found to precipitate at room temperature. Since we wished to retain the potassium phosphate incubation step in order to make these assays comparable in ionic strength and environment to our routine radiochemical assay for SSAO, the SDS/HCl step was therefore omitted. Instead it was found that the developed colour after ABTS/peroxidase addition remained unchanged for at least 1 hr if tubes were subsequently kept ice-cold, and absorbance readings were carried out at 414 nm in a Pye Unicam SP 500 Spectrophotometer within this time.

In order to study the effects of potential inhibitors 25 μl of inhibitor solution were preincubated (20 min, 37°) with 25 μl homogenate and 450 μl sodium azide/potassium phosphate buffer. In this case, assays were started by the addition of 25 μl 1 mM benzylamine or 0.5 mM MDL 72200.

Preliminary studies to produce calibration curves with standard H_2O_2 solutions yielded an estimated molar extinction coefficient for oxidized ABTS of $23{,}089 \pm 616\ M^{-1}\,cm^{-1}$ ($N = 4$ separate experiments). Specific enzyme activities (nmol H_2O_2 produced hr^{-1} mg protein^{-1}) were calculated using this value, as well as allowing for the fact that 1 molecule H_2O_2 oxidizes 2 molecules ABTS to 2 molecules radical cation [12].

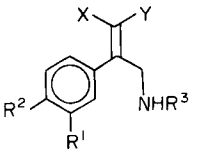
Protein concentrations of homogenates were estimated by the method of Lowry *et al.* [13].

RESULTS

Comparison of IC_{50} *values for inhibition of SSAO*

In the initial part of this study, the potencies of the various compounds to be tested as inhibitors of rat aorta SSAO were compared by the use of identical preincubation and assay conditions, and were expressed as estimated drug concentrations (IC_{50})

Table 1. Structural formulae and IC_{50} values for inhibition of rat aorta SSAO by 2-phenyl-allylamine derivatives



Compound (MDL No.)	R^1	R^2	R^3	X	Y	IC_{50} (nM)
72161	H	H	H	F	H	2.5
72274	H	H	H	Cl	H	8
72200	H	H	H	H	H	3000
72392	OH	H	H	F	H	20
72627	OH	H	H	Cl	H	60
72434	OH	OH	H	F	H	60
72486	OH	OH	H	H	F	350
72145*	OCH_3	OCH_3	H	F	H	6
72280	OCH_3	OCH_3	C_2H_5	F	H	100

* Data from ref. 9.

Ten-fold serial dilutions of drugs (usually 10^{-11}–10^{-3} M) were preincubated for 20 min at 37° with aliquots of aorta homogenate before assay of remaining SSAO activity with 1 μM [^{14}C]benzylamine. IC_{50} values represent drug concentrations required to inhibit control activities by 50% and were estimated from inhibition curves plotted with mean inhibition values, derived from triplicate assays on 3 separate homogenates studied with each drug.

required to inhibit control activities by 50% (Table 1).

The most potent compound in the series was (E)-2-phenyl-3-fluoroallylamine (MDL 72161) with an IC_{50} of 2.5 nM. Several derivatives of this compound with substituents on the phenyl ring were also examined (i.e. MDL 72392, 72434, 72145). The introduction of hydroxyl-substituents, at the 3-position (MDL 72392) and additionally at the 4-position of the ring (MDL 72434), resulted in progressively lower potency compared with MDL 72161. In contrast, the 3,4-dimethoxy derivative (MDL 72145) was only slightly weaker than MDL 72161. The decrease in potency occurring on introduction of hydroxyl groups in the phenyl ring was also demonstrated in the corresponding chloroallylamines (compare MDL 72274 with 72627).

The stereochemical position of the halogen atom and its identity also influenced inhibitory potency. The (E)-fluoro-derivatives (i.e. halogen *cis* relative to the benzene ring) were more potent (by approximately three-fold) than the corresponding (E)-chloro-compounds (compare MDL 72161 with 72274; 72392 with 72627). Comparison of two geometrical isomers (MDL 72434 and 72486) with the fluorine atom *cis* and *trans*, respectively relative to the aromatic ring, indicated that the *cis* arrangement is the more favourable. The absence of a halogen atom altogether (MDL 72200) resulted in a compound of relatively weak inhibitory activity. The last two compounds in Table 1 illustrate the effect of the substitution of the amine function. The primary amine MDL 72145 was approximately 17-times more potent than its corresponding (N-ethyl) secondary amine derivative (MDL 72280).

A previous study which used tyramine, a non-selective substrate for rat brain mitochondrial MAO-A and B, concluded that 2-phenyl-3-chloroallylamines are considerably weaker as MAO inhibitors than their corresponding 3-fluoro-analogues [2]. In view of the high potency of the chloroallylamine MDL 72274 against SSAO in the present study, its inhibitory activity was re-examined against rat liver MAO-A and B separately. IC_{50} values determined with 5-hydroxytryptamine and benzylamine, respectively, as specific substrates for MAO-A and B after 20 min preincubation of homogenates (N = 3) at 37° with ten-fold serial dilutions of drug were 11.7 μM (MAO-B) and 520 μM (MAO-A). In a previous study, the fluoroallylamine MDL 72145 gave values of 0.11 μM (MAO-B) and 2.8 μM (MAO-A) under similar conditions [9].

Dialysis experiments

It was found that the inhibitory effects of the allylamine lacking a halogen atom (MDL 72200) were completely reversed by dialysis. The effect of all the other compounds were barely, if at all, modified by dialysis indicating that they act predominantly as irreversible inhibitors of SSAO (data not shown).

Inhibition kinetics

Experiments were carried out to investigate the kinetic characteristics of the inhibition of SSAO produced by MDL 72145, MDL 72274 and MDL 72200. These studies indicated that the initial immediate inhibition of SSAO by the irreversible drugs MDL 72145 and MDL 72274 was competitive (representative individual experiments shown in Fig. 1). Estimated K_i values (mean ± SE of three experiments on different homogenates) were 630 ± 74 nM (MDL 72145) and 414 ± 44 nM (MDL 72274). Similar studies with the reversible inhibitor MDL 72200 yielded a K_i for competitive inhibition of 659 ± 10 nM (N = 3). The mean K_m value for [^{14}C]benzylamine as substrate in this overall series of experiments was 3.3 ± 0.4 μM (N = 9).

As found previously with MDL 72145 [9], the

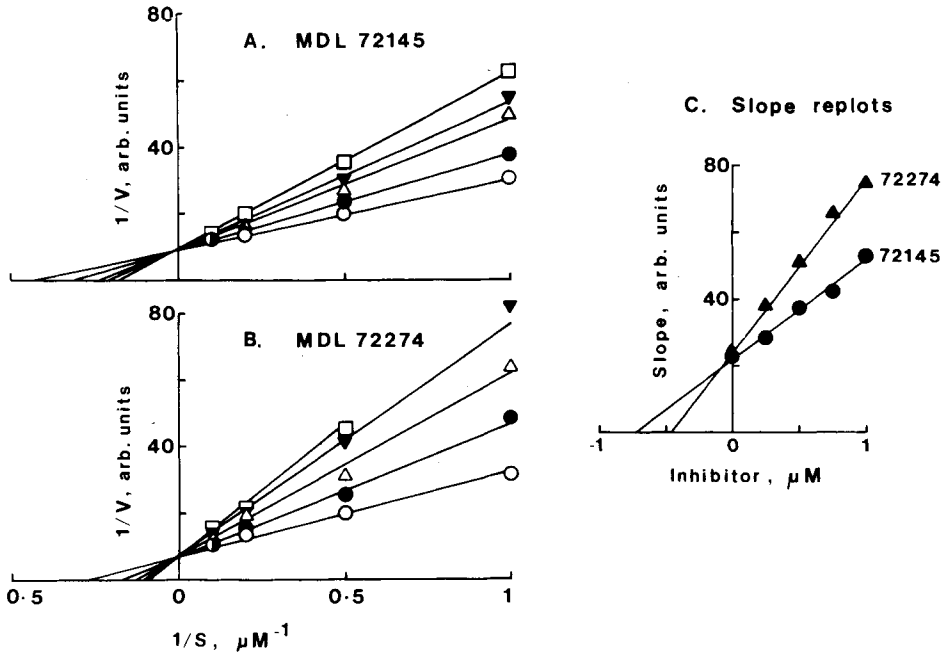


Fig. 1. Double reciprocal plots showing initial competitive inhibitory effects of MDL 72145 (panel A) and MDL 72274 (panel B) upon metabolism of [^{14}C]benzylamine (1–8 μM) in rat aorta homogenates (see text for details). Inhibitor concentrations were zero (○), 0.25 (●), 0.5 (△), 0.75 (▼) and 1 μM (□). Each point is the mean of triplicate assays. Results are from one experiment, representative of two others. Linear regression analysis of slope replots (panel C) in this particular experiment gave K_i values (from abscissa intercept) of 0.74 μM (MDL 72145) and 0.46 μM (MDL 72274).

inhibition of SSAO by MDL 72274 (10–100 nM) was increased with time if the drug was preincubated with aorta homogenates, before the subsequent addition of benzylamine to measure remaining enzyme activity. The half-life for enzyme inactivation during preincubation as before appeared to be directly proportional to the inverse of inhibitor concentration (data not shown). In contrast, the inhibition produced by MDL 72200 decreased with increasing preincubation time (Fig. 2). Subsequent

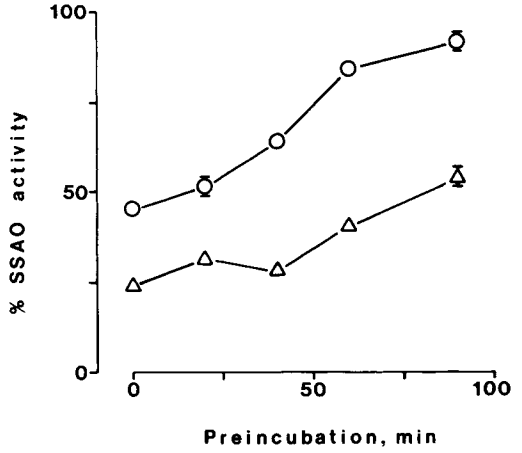


Fig. 2. Influence of preincubation time on inhibition of SSAO by MDL 72200. Drug concentrations at preincubation were 2 (○) and 5 (△) μM. Each point is the mean (± SE when exceeding symbol size) of triplicate assays on 3 separate aorta homogenates.

experiments (see below) indicated that this was due to the metabolism of this particular drug by SSAO.

Comparison of the metabolism of MDL 72200 and benzylamine by SSAO

The production of H_2O_2 arising from the deamination of MDL 72200 (1.25–25 μM) in aorta homogenates was estimated spectrophotometrically (see Materials and Methods). Comparisons on each aorta homogenate were also carried out with benzylamine (2.5–50 μM) as substrate.

At the bottom of these ranges of substrate concentrations (i.e. 1.25 and 2.5 μM) it was sometimes difficult to avoid a significant percentage (up to 50% with some homogenates) of available substrate being metabolised in the assay, as assessed by comparing measured H_2O_2 production with the total theoretical maximum for the particular substrate concentration present. This followed from the fact that the calculated maximum absorbance change for complete metabolism of 1.25 and 2.5 μM substrate in our assay was 0.029 and 0.058, respectively, whereas the minimum change in absorbance which could be estimated with precision above background was approx. 0.010. Consequently, assay incubation times were chosen to allow at least this minimum absorbance change to occur. Segel [14] has shown that under conditions where significant utilization of substrate occurs during enzyme assays, reliable kinetic constants can be estimated by means of a modified Lineweaver–Burk relationship in which [reaction product]$^{-1}$ is plotted against [mean substrate concentration]$^{-1}$, where the latter is the arithmetic mean of the initial con-

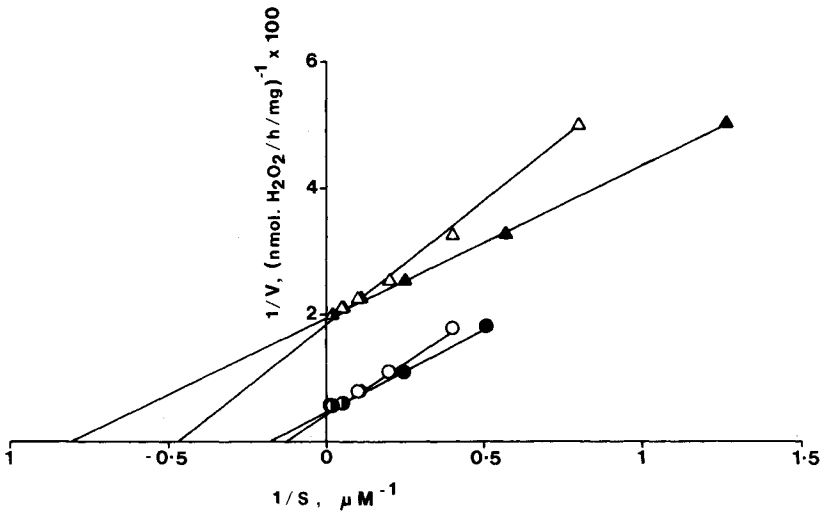


Fig. 3. Double reciprocal plots for metabolism of MDL 72200 (△, ▲) and benzylamine (○, ●) by the same rat aorta homogenate. Each point is the mean of duplicate determinations. Reaction velocities (V) are plotted either against initial substrate (S) concentrations (△, ○) or alternatively against mean substrate concentrations (▲, ●), the latter to "correct" for substrate depletion in the assay (see text). Lines are fitted by linear regression. The data is representative of two other experiments with different homogenates in which the substrates were also studied concurrently.

centration and the estimated final concentration of substrate at the end of the assay. Figure 3 shows representative data obtained on the same homogenate with benzylamine and MDL 72200 as substrates. The effect of using "mean substrate concentrations" (to "correct" the data) is compared with the use of initial substrate concentrations as "uncorrected" data in these plots. Overall kinetic constants (mean ± SE of three different homogenates) estimated by these methods were as follows: for benzylamine K_m (μM) = 10.4 ± 1.9 (uncorrected), 8.1 ± 1.9 (corrected), V_{max} (nmol H_2O_2 hr^{-1} mg $protein^{-1}$) = 244 ± 28 (uncorrected), 233 ± 26 (corrected); for MDL 72,200, K_m = 2.05 ± 0.10 (uncorrected), 1.40 ± 0.16 (corrected), V_{max} = 52 ± 6 (uncorrected), 50 ± 6 (corrected).

Various inhibitors were used in an attempt to identify the enzyme responsible for deamination of benzylamine and MDL 72200 in rat aorta. However, it was necessary first to ensure that any inhibitors included in the assay would not interfere with the peroxidase reaction which produces the oxidized ABTS. Thus, preliminary experiments examined possible drug effects upon the colour reaction produced from standard H_2O_2 solutions. It was found clorgyline (10^{-4} M), pargyline (10^{-3} M) or MDL 72274 (10^{-6} M) had no influence on colour formation. However, when 10^{-3} M semicarbazide or hydroxylamine were present with standard H_2O_2 solutions, on addition of ABTS/peroxidase, the usual characteristic blue colour formation occurred immediately but was rapidly bleached within seconds to render the resulting solution colourless, with a final absorbance at blank values. This suggests that these compounds do not prevent the instantaneous peroxidatic oxidation of ABTS, but that they do react in some way with the product immediately it is formed. Consequently, these particular carbonyl reagents, frequently used as SSAO inhibitors, cannot be utilized in this spectrophotometric assay.

For these reasons only clorgyline, pargyline and MDL 72274 at the doses above were included subsequently in assays with rat aorta homogenates. It was found that the MAO selective inhibitors clorgyline and pargyline had no effect on H_2O_2 production resulting from the metabolism of benzylamine (50 μM) or MDL 72200 (25 μM). In contrast, complete inhibition in both cases was obtained with MDL 72274, shown earlier in this paper to be a potent, selective SSAO inhibitor at 1 μM. Consequently, SSAO appears to be the amine oxidase responsible for benzylamine and MDL 72200 metabolism in the aorta homogenates.

DISCUSSION

Our results indicate that a number of 2-phenyl-3-haloallylamine derivatives are potent irreversible inhibitors of rat aorta SSAO. The inhibitory activity appeared to be enhanced by the presence of hydrophobic substituents on the aromatic ring, while the introduction of hydroxy-substituents in the ring reduced this activity. To some extent, these properties resemble the characteristics of arylamine substrates of SSAO in rat tissues, with hydrophobic compounds such as benzylamine, in particular, and β-phenylethylamine being metabolized with relatively low K_m values, whereas catecholamines such as dopamine, noradrenaline and adrenaline are metabolized poorly or apparently not at all by this enzyme (see ref. 7).

Irreversible inhibition of SSAO by these allylamine derivatives required the presence of a vinylic-halogen atom, preferentially in a *cis* position relative

to the aromatic moiety for maximum potency. This contrasts with the reversible inhibition demonstrated by MDL 72200 which lacks the relevant halogen.

The potency of MDL 72200 as a reversible inhibitor was found to decrease with increasing incubation time in the presence of aorta homogenates. After modification of a spectrophotometric assay, previously described for MAO activity [12], but used here to detect H_2O_2 formation from potential substrates of SSAO, we were able to demonstrate that MDL 72200 is metabolized by SSAO in aorta homogenates with a K_m of 1.4 μM. The K_i for competitive inhibition of [^{14}C]benzylamine metabolism by this compound was found to be 0.7 μM. The spectrophotometric assay used to determine the K_m for benzylamine metabolism yielded a value of 8.1 μM, which is close to the K_m values (around 2–5 μM) usually determined for benzylamine as substrate in the more sensitive radiochemical assays for SSAO. V_{max} values for MDL 72200 were approximately five times lower than those for benzylamine, indicating that the turnover of the former compound is much slower than benzylamine, despite the apparently higher affinity (i.e. lower K_m) for SSAO. We have previously demonstrated that benzylamine can be used as a substrate in a histochemical staining method to localize SSAO in smooth muscle of rat aorta [5]. In preliminary studies we have found that MDL 72200 can also be used to produce this staining which, in turn, could be prevented by preincubation of aorta sections with 1 mM semicarbazide or 1 μM MDL 72274 (unpublished results). As a whole, our data showing MDL 72200 to be a substrate for SSAO is consistent with reports elsewhere that allylamine itself is also metabolized by SSAO in homogenates of rat and human cardiovascular tissues [15, 16].

In an earlier study, the kinetics of irreversible inhibition of SSAO by the fluoroallylamine derivative MDL 72145 appeared to be consistent with a simple bimolecular reaction between drug and enzyme [9]. However, by studying the kinetics of the initial interaction, we have now found evidence for an early competitive binding step with SSAO for both MDL 72145 and the chloroallylamine MDL 72274, with K_i values of around 0.4–0.6 μM. These results are therefore consistent with a mechanism in which the allylamine inhibitor (I) and the enzyme (E) combine to form a reversible complex (EI) as an intermediate before the irreversible complex (EI*) is produced, as shown below.

$$E + I \rightleftharpoons EI \rightarrow EI^*$$

Our previous evidence for a bimolecular irreversible reaction between MDL 72145 and SSAO was based on finding an apparent first-order relationship between the half-life ($t_{1/2}$) for enzyme inactivation during preincubation and the inverse of inhibitor concentration [I] using the analytical method described by Kitz and Wilson [17]. A similar result was found in the current studies with MDL 72274. However, these experiments were only carried out with inhibitor concentrations from 10 to 100 nM, which are well below the K_i values estimated by the Lineweaver–Burk analysis for the reversible binding step. In the Kitz and Wilson analysis, the use of very low concentrations relative to the true K_i can theoretically produce a plot of $t_{1/2}$ vs $[I]^{-1}$ passing so close to the origin, that the intercepts on the axes predicted for a mechanism showing saturation kinetics, may be difficult or even virtually impossible to observe under these conditions. This may be the explanation for our original erroneous conclusions about the inhibitory mechanism obeyed. We did attempt to carry out further studies with inhibitor concentrations above 100 nM. However, the major problem which arose here was the need to employ very large dilutions of enzyme–inhibitor mixtures after preincubation in order to reduce the inhibitor concentration enough to prevent any reversible inhibition being apparent at zero preincubation time, and also to prevent further irreversible inhibition developing in the presence of excess substrate (here 10 μM benzylamine) in the assay. With these larger dilutions, the homogenates became too weak to allow reliable determination of enzyme activity. Consequently, our attempts to use the methods of Kitz and Wilson [17] to confirm the two-step mechanism for irreversible inhibition shown above have so far been unsuccessful.

The inhibition of MAO by several of the compounds used here has previously been described [2–4]. In general, fluoroallylamines were potent inhibitors of tyramine deamination in rat brain (IC_{50} values from 10^{-9}–10^{-6} M) whereas the chloroallylamine MDL 72274 was much weaker (IC_{50} of 10^{-4} M). With the use of the selective substrates 5-hydroxytryptamine and benzylamine, respectively for MAO-A and B in rat liver, MDL 72274 was found to be more active against MAO-B (IC_{50} of 11.7 μM) than against MAO-A (IC_{50} of 520 μM) in the present study. However, it is also clear that the drug is considerably more potent (IC_{50} of 8 nM) as an inhibitor of SSAO suggesting that MDL 72274 could be a useful pharmacological tool for investigating the poorly understood physiological importance of SSAO in animal tissues. Consistent with the *in vitro* data shown here, *ex vivo* inhibition of SSAO is also found in tissue homogenates after administration of MDL 72145 [18] or MDL 72274 [11] to rats at doses similar to or lower than those required to inhibit MAO, respectively.

In conclusion, the irreversible inhibition of SSAO is a significant pharmacological property of the 2-phenyl-3-haloallylamines, originally developed and currently under investigation as potential MAO inhibitors. The clinical relevance of SSAO inhibition which may accompany the use of these and other drugs is unclear while the physiological role of this enzyme in vascular and other sites remains unknown. However, the identification of highly potent and apparently selective agents for SSAO, such as MDL 72274 in this study may well be exploitable in future endeavours.

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