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An allylamine derivative (MDL 72145) with potent irreversible inhibitory actions on rat aorta semicarbazide-sensitive amine oxidase

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(E)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) was found to be an extremely potent inhibitor of the semicarbazide-sensitive amine oxidase (SSAO) in rat aorta homogenates. Considerable inhibition, which was not reversed by dialysis, could be produced under appropriate in-vitro conditions at drug concentrations around 10 nM. The pseudo first order kinetics for time-dependent inhibition by MDL 72145 (10–100 nM) were found to be consistent with a bimolecular reaction between enzyme and inhibitor with a rate constant for this step of 2×10^6 min^{-1} M^{-1}. A similar rate of inhibition under an oxygen atmosphere to that obtained under nitrogen was produced upon incubation of enzyme with inhibitor, suggesting that oxidation of the inhibitor to an active metabolite was not required for its activity. Incubation of homogenates for very short periods (1 min) with inhibitor (0·05–0·5 μM) and benzylamine (1–10 μM) as substrate indicated non-competitive kinetics for the early interaction of enzyme with the drug. Benzylamine (50 μM), but not pyridoxal phosphate (100 μM), was able to protect SSAO from inhibition by 10 nM MDL 72145. However, pyridoxal phosphate alone appeared to produce some irreversible inhibition of the enzyme. Dialysis against buffer containing 50 μM or 1 mM benzylamine was unable to reactivate SSAO inhibited by 10 nM MDL 72145. It is concluded that MDL 72145 irreversibly inhibits SSAO by acting at, or near, the substrate binding site, but the exact nature of the complex formed remains to be identified.

There is now considerable evidence that the outer mitochondrial membrane enzyme monoamine oxidase (MAO) exists in two forms (MAO-A and MAO-B) which differ with respect to their substrate specificities and also their sensitivities towards a variety of inhibitor drugs, including acetylenic compounds such as clorgyline and (−)-deprenyl (selegiline) (see Fowler et al 1981, for review).

Recently, a new group of MAO inhibitors derived from the unsaturated aliphatic amine, allylamine has been described. One particular compound, MDL 72145 ((E)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine) is an irreversible MAO-B selective inhibitor. The drug is relatively inactive at potentiating the cardiovascular effects of tyramine (Bey et al 1984) and therefore appears less likely to provoke the 'cheese reaction' with dietary amines, which is shown by many other MAO inhibitors (Blackwell 1963).

In addition to the flavoprotein MAO, many animal tissues also contain a semicarbazide-sensitive amine oxidase (SSAO) which may belong to the group of enzymes with pyridoxal phosphate as their cofactor (Blaschko 1974). The physiological importance of SSAO is unknown. However, its relatively high activity in blood vessel homogenates (Lewinsohn et al 1978), and its localization by histochemical methods to the smooth muscle layers of blood vessels (Ryder et al 1979) may indicate that in some tissues, at least, the enzyme may be involved in some undefined aspect of vascular function.

In the rat, SSAO may be distinguished from MAO by having a very low K_m (around 5 μM) towards benzylamine as substrate. Also, SSAO is virtually unaffected by acetylenic inhibitors such as clorgyline at concentrations (around 10^{-3} M) sufficient to inhibit MAO activities completely. In contrast, the enzyme is inhibited by 10^{-3} M semicarbazide, which has little or no activity against MAO at this concentration. Some other hydrazine-based drugs, such as phenelzine, hydralazine and benserazide are inhibitors of both SSAO and MAO, although some differences in their relative potencies and their inhibitory properties towards these enzymes have also been characterized (see Lyles 1984 for review).

Nelson & Boor (1982) recently showed that SSAO in rat aorta homogenates can metabolize allylamine to the toxic aldehyde acrolein, and that the latter compound may be responsible for the cardiovascular

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necrosis following allylamine administration to rats. By behaving as an alternative substrate for SSAO, allylamine also acted as a competitive inhibitor of in-vitro benzylamine metabolism by the enzyme. For these reasons, it was of interest that interactions between SSAO and the allylamine derivative MDL 72145 might also occur. In this paper we report that MDL 72145 is an extremely potent, irreversible inhibitor of SSAO in rat aorta homogenates and furthermore, this inhibition appears unlikely to require the oxidative deamination of the compound to a reactive metabolite.

MATERIALS AND METHODS

Materials

[Methylene-^{14}C] benzylamine hydrochloride ([^{14}C]Bz) was purchased from the Radiochemical Centre (Amersham, UK). Unlabelled benzylamine hydrochloride was obtained from Sigma (London) Chemical Co. (Poole, UK).

The hydrochloride of MDL 72145 was a gift from Merrell Dow Research Institute, Strasbourg, France.

Adult male or female Wistar rats (200–400 g) were obtained from our Departmental breeding colony, Animal Services Unit, University of Dundee.

Methods

Rats were killed by cervical dislocation and aortae were dissected to include both thoracic and abdominal parts of the vessel. The aortae were weighed, and then homogenized in a ground-glass homogenizer, in 1 mM potassium phosphate buffer, pH 7·8 usually at a tissue (g) : buffer (ml) ratio of 1 : 40 unless indicated otherwise. After centrifugation of the homogenate at 600*g* for 10 min, the supernatant was removed and diluted further by eight-fold with the homogenization buffer, before use in the studies below.

For most inhibition studies, 25 µl homogenate samples were preincubated in air at 37 °C with 25 µl volumes of appropriate MDL 72145 solutions (prepared by dissolving the hydrochloride in 0·2 M potassium phosphate, pH 7·8). All drug concentrations quoted in the text refer to *preincubation* concentrations unless indicated otherwise. Control homogenates were preincubated with buffer lacking the drug. After appropriate preincubation periods, remaining SSAO activity was assayed, as previously described in detail (Lyles & Callingham 1982), by incubation for 5 min at 37 °C with a final benzylamine concentration of 1 µM in the assay. This was obtained by the addition of 50 µl 2 µM [^{14}C]Bz (sp. act. 10 µCi µmol^{-1} in 0·2 M potassium phosphate buffer, pH 7·8) to each preincubation mixture.

For dialysis studies, mixtures containing 0·5 ml of aorta homogenate and 0·5 ml of appropriate drug or buffer solutions (for controls) were preincubated and then dialysed under appropriate conditions (see Results) for at least 18 h. SSAO activity in 50 µl aliquots of each dialysed sample was then assayed by addition of 50 µl of 2 µM [^{14}C]Bz, as described above.

The time-dependence and kinetics of irreversible inhibition were studied by the methods of Kitz & Wilson (1962). Homogenates for these experiments were prepared at a tissue (g) : buffer (ml) ratio of approx. 1 : 30 and after centrifugation as above, they were used without further dilution. Mixtures containing homogenate (0·3 ml) and appropriate MDL 72145 solutions (0·3 ml) to give inhibitor concentrations from 10–100 nM were set up in ice-cold vessels and then preincubated for various periods at 37 °C. After the appropriate times, 100 µl of each mixture were diluted by addition to 4·9 ml ice-cold 0·1 M potassium phosphate buffer, pH 7·8. Preliminary experiments showed that little, if any, time-dependent inhibition of enzyme activity is produced by incubating 10–100 nM drug with homogenates in ice-cold vessels for periods up to 1 h. SSAO activity after dilution was assayed by incubation of 50 µl diluted sample with 50 µl of 20 µM [^{14}C]Bz for 30 min. This procedure thus resulted in a 100-fold dilution of inhibitor (and enzyme) from the preincubation to the final assay mixture.

The kinetics and type of inhibition apparent after very short incubation times was studied by incubating inhibitor (25 µl), homogenate (25 µl) and [^{14}C]Bz (50 µl) solutions for 1 min at 37 °C. Final MDL 72145 concentrations were 50–500 nM and substrate (Bz) concentrations were 1–10 µM. In order to ensure adequate measureable metabolite production during these short incubation times, it was necessary to use 1 : 40 homogenates (see above) without further dilution after centrifugation.

Protein concentrations of aorta homogenates were assayed by the method of Lowry et al (1951).

The specific enzyme activities of all the control homogenates used in these experiments ranged between 20·3 and 62·9 nmol (mg prot.)$^{-1}$ h^{-1}. The range of activities found may reflect the use of animals from either sex and of varying age in these experiments although these factors were not studied specifically here. For example, the specific activity of aortic SSAO has been reported to decrease with age (Cao Danh et al 1984). However, there was no indication that the inhibitory properties of MDL

72145 were modified by either the sex or the age of the animal. All experiments were replicated with different aortae to ensure consistency of results, and representative results on individual aorta homogenates are presented below when appropriate.

RESULTS

Preincubation time-dependence of SSAO inhibition by MDL 72145

In preliminary experiments to investigate the possibility of SSAO inhibition by MDL 72145, samples of rat aorta homogenate were preincubated for 20 min at 37 °C with various concentrations (10^{-9}–10^{-6} M) of the drug. Under these conditions, enzyme activity was completely inhibited at 10^{-7} M with an estimated IC50 of around 6 nM (Fig. 1).

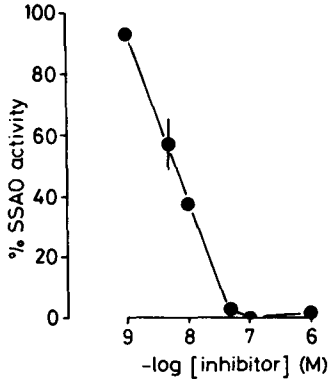

FIG. 1. Inhibition of rat aorta SSAO by MDL 72145. Homogenate samples were preincubated with the drug for 20 min at 37 °C before assay of remaining SSAO activity which is expressed as a percentage of the activity in control samples preincubated without inhibitor. Each point is the mean (and range when exceeding symbol size) of triplicate assays on three different aortae, except for the points 5×10^{-9} and 5×10^{-8} M where two aortae were used.

The inhibition was subsequently found to be dependent upon preincubation time. Since dialysis (see below) indicated that MDL 72145 is an irreversible inhibitor of SSAO, this time-dependence was studied by the methods of Kitz & Wilson (1962). Fig. 2 shows a representative experiment in which the kinetics were found to be consistent with a first-order inhibition process with similar results being obtained in other experiments. The replot of the half-life ($t_{\frac{1}{2}}$) for enzyme inhibition against the reciprocal of corresponding inhibitor (I) concentration is also shown. A linear relationship through the origin was obtained. For pseudo first-order kinetics resulting from a bimolecular inhibition mechanism (see Discussion), the slope of this plot has a value of $(\log_e 2) \times k^{-1}$ where k is the bimolecular rate constant for irreversible inhibition. From experiments on 3 different aortae, k was found to be $2{\cdot}0 \pm 0{\cdot}1 \times 10^6$ min^{-1} M^{-1} (mean ± s.e.).

The kinetic nature of the initial interactions between inhibitor and enzyme was investigated by incubating them together with benzylamine (as substrate) for very short assay periods. In these experiments in which no preincubation of inhibitor with enzyme occurred, the resulting double reciprocal plot indicated a non-competitive kinetic mechanism for the inhibition (Fig. 3).

The possibility that oxidation of the inhibitor by the enzyme was required for the inhibitory effects was investigated by preincubating 10 nM MDL 72145 with aorta homogenates after extensively flushing assay tubes containing drug-enzyme mixtures with either nitrogen or oxygen, followed by sealing the tubes with rubber stoppers to maintain the required gas phase. There was no difference in the rate of enzyme inhibition under the two different gaseous environments (not shown).

Dialysis studies

Other investigations into the inhibitory characteristics of the drug involved dialysis techniques. Table 1 shows the effects of dialysis against 1 litre of 0·1 M potassium phosphate buffer pH 7·8 at either 4 °C or 25 °C upon enzyme inhibited by preincubation with 10 nM MDL 72145. Dialysis was continued for 18 h with a single change of buffer after about 2 h. SSAO activity in inhibitor-treated samples was 22 and 25% of control samples dialysed similarly at 4 and 25 °C, respectively. These values compared with 8% of control activity remaining in inhibited samples which were not dialysed, but instead stored concurrently during the dialysis period at the appropriate temperature. These results indicate that inhibition of SSAO by MDL 72145 is largely resistant to reversal by dialysis, although the possibility of a slight reversal of inhibition is raised by the existence of a greater degree of inhibition in the undialysed drug-treated samples. However, subsequent experiments indicated that the latter observations may be due, in fact, to an additional slow increase in enzyme inhibition which develops during the storage of the undialysed samples and becomes superimposed upon the more rapid inhibition occurring during the initial preincubation period (at 37 °C). For example, when aorta homogenates were incubated for up to 24 h at 4 °C with 10 nM MDL 72145 the following activities (% of controls) after various incubation times (in parentheses) were obtained: 84·9% (1 h),

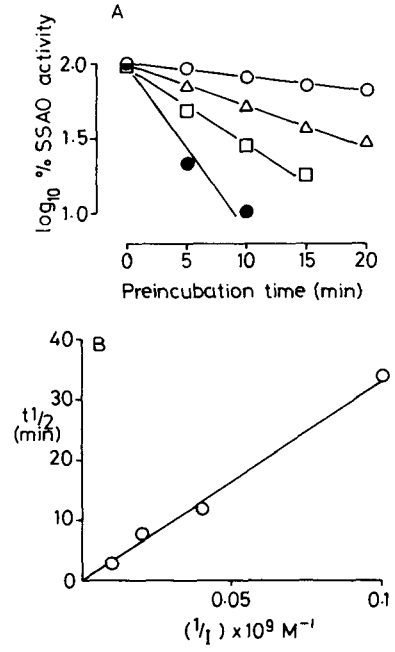

FIG. 2. A: Preincubation time-dependent inhibition of rat aorta SSAO by 10 (○), 25 (△), 50 (□) and 100 nM (●) MDL 72145. Each point is the mean of triplicate determinations expressed as a percentage of corresponding control samples preincubated without inhibitor. B: Replot of half-lives ($t_{\frac{1}{2}}$) for enzyme inactivation and reciprocal of inhibitor (I) concentration.

76·4% (2 h), 73·4% (3 h), 59·8% (4 h) and 0·9% (24 h).

Subsequent experiments were designed to investigate whether the presence of benzylamine (50 μM) or pyridoxal phosphate (100 μM) in the preincubation mixtures could protect SSAO from inhibition by MDL 72145. After preincubation, samples were dialysed for 18 h at 4 °C (as above). Table 2 shows the results of two representative experiments. In Experiment 1, preincubation with benzylamine alone resulted in no effect after dialysis upon SSAO activity, whereas approximately 90% inhibition by MDL 72145 was produced. This was reduced to only 26% inhibition in samples originally preincubated with both benzylamine and inhibitor, indicating a marked protective effect of benzylamine. In contrast, in Experiment 2 pyridoxal phosphate had little if any protective action against inhibition by MDL 72145. However, it should be noted that preincubation with pyridoxal phosphate alone resulted in 37% inhibition of enzyme activity after the dialysis.

In final experiments the possibility was considered that dialysis of inhibited enzyme against buffers containing benzylamine could reactivate the enzyme. Here, samples inhibited by preincubation with 10 nM MDL 72145 were dialysed firstly for 24 h against 0·1 M potassium phosphate buffer, pH 7·8, containing either 50 μM or 1 mM benzylamine, and then secondly, for 18 h against the buffer alone to remove the unlabelled benzylamine before enzyme assays with [^{14}C]Bz. Table 3 shows that neither benzylamine concentration during dialysis was capable of reversing the inhibition of SSAO by MDL 72145.

DISCUSSION

The results presented here indicate that MDL 72145 is an extremely potent, irreversible inhibitor of SSAO in rat aorta homogenates. This conclusion is based upon the inability of dialysis to reverse the inhibition, and also upon the preincubation time-dependence for the inhibitory actions.

In general, irreversible inhibitors may act via two kinetically distinguishable types of mechanism (see Tipton 1980) shown below.

(1) $E + I \xrightarrow{k} EI^*$

(2) $E + I \overset{K_i}{\rightleftharpoons} EI \xrightarrow{k_2} EI^*$

Mechanism (1) involves a bimolecular reaction between enzyme (E) and inhibitor [I], and will produce pseudo first-order kinetics if [I] ≫ [E]. In mechanism (2), irreversible complex (EI*) formation depends on the prior formation of the reversible complex (EI). Here, first order kinetics is produced if k_2 is small and does not prevent EI from reaching equilibrium. Plots of half-life ($t_{\frac{1}{2}}$) vs $[I]^{-1}$ for the first

Table 1. Effects of dialysis at 4 and 25 °C on the inhibition of rat aorta SSAO by MDL 72145 (10 nM). Samples were preincubated for 60 min at 37 °C without (controls) and with 10 nM MDL 72145. Some samples were dialysed for 18 h at either 4 or 25 °C. Undialysed samples were stored for the same time at the appropriate temperature. SSAO activity was assayed (in triplicate) in each sample with 1 μM [^{14}C]Bz. Each value is the mean ± s.e. of determinations on three samples in each category, all originally derived from the same rat aorta homogenate. Figures in parentheses represent percentage activities of samples expressed relative to the appropriate controls subjected to the same experimental protocol.

	SSAO activity (nmol (mg prot.)$^{-1}$ h^{-1})	
Sample	4 °C	25 °C
Undialysed:		
Control	30·9 ± 1·1 (100)	30·9 ± 1·2 (100)
MDL 72145	2·5 ± 0·3 (8)	2·5 ± 0·5 (8)
Dialysed:		
Control	34·2 ± 4·1 (100)	31·4 ± 0·4 (100)
MDL 72145	7·4 ± 0·5 (22)	7·7 ± 0·6 (25)

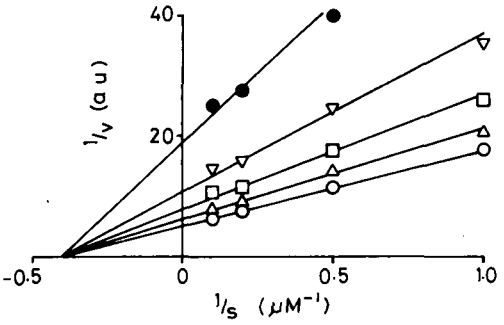

FIG. 3. Double reciprocal plot for inhibition of rat aorta SSAO by zero (○), 50 (△), 125 (□), 250 (▽) and 500 nM (●) MDL 72145. Initial reaction velocities (V) in arbitrary units (au) were measured after 1 min incubation with different substrate (S) concentrations of benzylamine. Each point is the mean of triplicate determinations.

order inhibition data will distinguish between the two mechanisms, since a line through the origin is predicted by (1), whereas a linear relation with intercepts on both axes enable K_i and k_2 to be determined in mechanism (2) (e.g. Kitz & Wilson 1962; Tipton 1980).

The present data suggest that mechanism (1) accounts for the kinetics for inhibition of SSAO by MDL 72145. The lack of any obvious intercept effects in the replots of Fig. 2 indicates that if the drug is capable of forming reversible complexes with SSAO, they are probably kinetically insignificant in determining the rate of irreversible enzyme inhibition by the range of inhibitor concentrations studied.

Table 2. SSAO activity in rat aorta homogenates dialysed after treatment with benzylamine (50 μM), pyridoxal phosphate (100 μM) or MDL 72145 (10 nM). Samples were preincubated for 60 min at 37 °C with the appropriate agents. They were then dialysed for 18 h at 4 °C followed by assay (in triplicate) of SSAO activity in each sample with 1 μM [^{14}C]Bz. Each value is the mean ± s.e. of determinations on three samples in each category derived within each experiment from the same rat aorta homogenate. Figures in parentheses represent the percentage activities of samples expressed relative to the appropriate controls.

Sample	SSAO activity nmol (mg prot.)$^{-1}$ h^{-1}
Expt. 1:	
Control	62·9 ± 3·6 (100)
Benzylamine	61·1 ± 2·2 (97)
MDL 72145	7·1 ± 0·7 (11)
Benz. + MDL	46·7 ± 1·2 (74)
Expt. 2:	
Control	58·8 ± 1·8 (100)
Pyridoxal phosphate (PP)	36·8 ± 0·3 (63)
MDL 72145	5·0 ± 0·1 (9)
PP + MDL	8·1 ± 0·2 (13)

Table 3. Effects of dialysis against benzylamine-containing (Bz) buffers upon inhibition of rat aorta SSAO by MDL 72145 (10 nM). Samples were preincubated for 60 min at 37 °C without (controls) or with MDL 72145. Samples labelled 'Bz' below were dialysed for 24 h at 4 °C against buffer containing benzylamine, followed by dialysis for a further 18 h against buffer alone. Other samples were dialysed for the complete period against buffer only. All were assayed in triplicate for SSAO activity with 1 μM [^{14}C]Bz. Each value is the mean ± s.e. of determinations on three samples in each category derived within each experiment from the same rat aorta homogenate. Figures in parentheses represent percentage activities of samples expressed relative to the appropriate controls.

Sample	SSAO activity nmol (mg prot.)$^{-1}$ h^{-1}
Expt. 1	
Control/no Bz	51·8 ± 0·3 (100)
Control/Bz (50 μM)	52·2 ± 0·6 (101)
MDL 72145/no Bz	8·3 ± 0·7 (16)
MDL/Bz (50 μM)	10·3 ± 0·9 (20)
Expt. 2	
Control/no Bz	20·3 ± 1·7 (100)
Control/Bz (1 mM)	21·0 ± 0·6 (103)
MDL 72145/no Bz	1·8 ± 0·3 (9)
MDL/Bz (1 mM)	1·9 ± 0·2 (9)

It was not possible to use higher inhibitor concentrations in this type of experiment, since it was found that the dilution procedure was no longer sufficient to reduce the inhibitor concentration enough to prevent further inhibition occurring during the assay incubation. This technical difficulty could not be satisfactorily overcome by using even greater dilutions, since this introduced unacceptable imprecision in determining SSAO activities in the extremely dilute homogenates.

However, it was possible to examine the initial kinetic effects of higher MDL 72145 concentrations (50–500 nM) by incubation of inhibitor with enzyme and benzylamine as substrate. Here, non-competitive inhibition occurred after extremely short incubation times. Thus, in this type of experiment, the inability to observe competitive, or even mixed-type, inhibition again argues against a significant early reversible complex formation between drug and enzyme. Overall, the indication from our experiments that inhibition of SSAO follows mechanism (1) above, is in contrast to results with MAO-A and -B, which suggest that mechanism (2) is more consistent with inhibition of the latter enzymes (Bey et al 1984).

Allylamine is believed to undergo oxidative deamination by SSAO to produce the toxic aldehyde acrolein and it has been suggested that the non-specific reactivity of this metabolite towards many

cellular constituents may be responsible for the cardiovascular necrosis produced by allylamine administration to the rat (Nelson & Boor 1982). Our studies, with MDL 72145 suggest that metabolism to its corresponding aldehyde is unlikely to be responsible for irreversible inhibition of SSAO since preincubation under nitrogen did not reduce the rate of enzyme inactivation. Although SSAO activity is not as sensitive as MAO activities to changes in oxygen tension, nevertheless significantly different rates of substrate (benzylamine) metabolism have been demonstrated in comparison of catalysis in assays flushed extensively with oxygen and nitrogen, as in the current experiments (Clarke et al 1982; Andree & Clarke 1982a). In further studies, dialysis at 25 °C of inhibited enzyme samples was used to investigate the possibility that reactivation of enzyme may occur by slow metabolism of the bound inhibitor, as demonstrated for the hydrazine-based SSAO inhibitor phenelzine (Andree & Clarke 1982b). However, in the current studies with MDL 72145, no evidence was obtained for any greater recovery of enzyme activity after dialysis at 25 °C than at 4 °C. Thus, although our data are not consistent with significant oxidation of MDL 72145 to an inhibitory metabolite, nevertheless it is possible that enzymatic modification of the drug to a non-oxidized intermediate could be responsible for the inhibitory effects described in this paper. Whatever the mechanism, it appears that the drug binds at or near the active site of the enzyme since protection from inhibition was afforded by benzylamine at a concentration (50 μM) about ten-fold higher than its K_m for metabolism (Lyles & Callingham 1975; Clarke et al 1982).

The sensitivity of SSAO to inhibition by carbonyl reagents has suggested that pyridoxal phosphate may be the enzyme cofactor (see Lyles 1984). In the present experiments, pyridoxal phosphate (100 μM) was unable to prevent SSAO inhibition by MDL 72145 indicating that an interaction of the drug with the putative cofactor is unlikely. Surprisingly, the enzyme activities of dialysed aorta homogenates, preincubated previously with pyridoxal phosphate alone, indicated that pyridoxal phosphate itself produces irreversible inhibition of the enzyme and this finding was confirmed in other experiments (not shown). Although we have no explanation for these effects at the present time, it is interesting that some indications of SSAO inhibition by this agent have been observed before (Lyles et al 1983) and the unequivocal proof of pyridoxal phosphate as cofactor remains to be established.

Rando & Eigner (1977) reported that the pseudo-irreversible inhibition of MAO by allylamine could be reversed by incubation of inhibited enzyme with benzylamine. It was proposed that the substrate could react with the bound inhibitor to remove it from the enzyme. In contrast, Bey et al (1984) found no reactivation by benzylamine of MAO activity inhibited by MDL 72145. In our studies, we also found no reactivation of SSAO after prolonged dialysis against solutions containing either 50 μM or 1 mM benzylamine.

In summary, the allylamine derivative MDL 72145 represents a structurally new class of potent inhibitor for SSAO. Its action appears to involve irreversible and presumably covalent binding at the substrate binding site of the enzyme. It is of interest that, qualitatively speaking, irreversible inhibition of SSAO by MDL 72145 was produced in the current experiments by drug concentrations rather lower than those required to produce comparable irreversible inhibition of rat brain MAO activities (Bey et al 1984). In separate experiments in which MDL 72145 was preincubated for 20 min with rat liver homogenates, we have found mean IC50 values (n = 3) for rat liver MAO-A (assayed with 100 μM 5-hydroxytryptamine) and MAO-B (assayed with 100 μM benzylamine) of $2{\cdot}8 \times 10^{-6}$ M and $1{\cdot}1 \times 10^{-7}$ M, respectively (unpublished data). Of course, the duration of preincubation, the enzyme concentration in the assay and also differences in the kinetic mechanisms obeyed will all influence the final level of inhibition and thus the apparent potencies of this drug upon the different amine oxidases. Nevertheless, it would appear that significant inhibition of SSAO may be produced in-vitro and possibly in-vivo as well, by doses of MDL 72145 being studied for its MAO-B selective properties. At present, the chemical nature of the complex between SSAO and MDL 72145 is unknown. However, a number of active site directed irreversible enzyme inhibitors containing the fluorine atom are now known (e.g. Jung et al 1980), and the possible influence of the fluorine moiety in the inhibition of SSAO by MDL 72145 remains to be investigated.

The potential importance of SSAO inhibition by this and other drugs remains unclear. However, the ability of the enzyme to deaminate certain trace amines, as well as its high activity in vascular smooth muscle and its probable association with the cell plasmalemma (see Lyles 1984 for review) all suggest possible future avenues for investigating the unknown physiological and pharmacological relevance of this enzyme.

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