Biochemical Pharmacology, Vol. 43, No. 2, pp. 307–312, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

INHIBITION OF A TYPE B MONOAMINE OXIDASE INHIBITOR, (*E*)-2-(4-FLUOROPHENETHYL)-3-FLUOROALLYLAMINE (MDL-72974A), ON SEMICARBAZIDE-SENSITIVE AMINE OXIDASES ISOLATED FROM VASCULAR TISSUES AND SERA OF DIFFERENT SPECIES

PETER H. YU* and DONG-MEI ZUO

Neuropsychiatric Research Unit, Department of Psychiatry, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0, Canada

(*Received* 24 *June* 1991; *accepted* 5 *September* 1991)

Abstract—(*E*)-2-(4-Fluorophenethyl)-3-fluoroallylamine hydrochloride (MDL-72974A) has been discovered recently to be a very potent and highly selective type B monoamine oxidase inhibitor. We have found that this inhibitor is also capable of inhibiting semicarbazide-sensitive amine oxidases (SSAOs) obtained from vascular tissues and sera of different species. The inhibition of SSAO by MDL-72974A was irreversible and time dependent. It was competitive without preincubation of the enzyme with the inhibitor and demonstrated a mixed-type of inhibition when the enzyme was preincubated with the inhibitor. The IC_{50} values were estimated to be 2×10^{-9} M, 5×10^{-9} M, 8×10^{-8} M and 2×10^{-8} M for SSAO from dog aorta, rat aorta, bovine aorta and human umbilical artery, respectively. SSAO obtained from bovine serum was relatively insensitive to MDL-72974A ($IC_{50} = 3 \times 10^{-7}$ M. Following intraperitoneal administration of MDL-72974A, rat brain MAO-B was inhibited with the ED_{50} value being about 0.2 mg/kg. Rat aorta SSAO was also inhibited and to a similar extent by the same dose. MDL-72974A is the most potent SSAO inhibitor that has been described thus far.

Semicarbazide-sensitive amine oxidase (SSAO,† EC 1.4.3.6) is an enzyme or group of enzymes residing predominantly in the plasma membrane of vascular smooth muscle cells, such as in blood vessels, heart tissue [1–3], as well as rat bone [4], interscapular brown adipose tissue [5], small intestine [6], cartilage [7], and human uterus [8]. It catalyzes the deamination of monoamines as does monoamine oxidase (MAO), but it is distinctly different from MAO. Monoamine oxidases (MAO-A and MAO-B) are well known to be flavine-containing enzymes located on the outer membrane of mitochondria in almost all mammalian tissues [9]. SSAO has been considered thus far to be a pyridoxal phosphate (PLP)- [10] or pyrroloquinoline quinone (PQQ)- [11] dependent copper enzyme. SSAO is inhibited by hydrazine compounds but is insensitive to MAO inhibitors, such as the propargylamine compound clorgyline [12]. SSAO can readily oxidize aliphatic amines including methylamine [13–15]. MAO can also deaminate aliphatic amines, but only when they have a chain length of 3 carbons or more [16].

The physiological functions of SSAO are not well understood. The high SSAO activity found in cardiovascular tissues suggests that it may play an important role in these tissues. It is involved in the deamination of circulating biogenic amines [17], and it has been established that SSAO is involved in the bioconversion of allylamine, an industrial chemical, which causes extensive and progressive vascular and myocardial lesions in several mammalian species [18].

Although hydrazine compounds, such as semicarbazide, are able to inhibit SSAO activity, they are rather nonspecific and relatively weak inhibitors, i.e. with an IC_{50} of about 10 μM. Recently, a new, highly potent, MAO-B inhibitor (*E*)-2-(4-fluorophenethyl)-3-fluoroallylamine hydrochloride (MDL-72974) (MDL-72974 is identical to MDL-72974A which is the hydrochloride salt) has been discovered [19]. It is much more potent that deprenyl, an MAO-B inhibitor with proven efficacy in the treatment of Parkinsonism [20]. In this study we have shown that MDL-72974A is also a potent SSAO inhibitor both *in vitro* and *in vivo* in several species including SSAO from human umbilical artery.

MATERIALS AND METHODS

Materials. Aortae were collected from Wistar male rats (200 g), Canadian huskie dogs, and bovine (Intercontinental Packers, Saskatoon). Human umbilical artery was provided by the Department of Obstetrics and Gynecology, Royal University Hospital, Saskatoon. Benzylamine, methylamine, homovanillic acid, bovine serum amine oxidase

* Corresponding author: Dr. Peter H. Yu, Neuropsychiatric Research Unit, Department of Psychiatry, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0, Canada. Tel. (306) 966-8816; FAX (306) 966-8830.

† Abbreviations: SSAO, semicarbazide-sensitive amine oxidase; MAO, monoamine oxidase; BSAO, bovine serum amine oxidase; MDL-72974A, (*E*)-2-(4-fluorophenethyl)-3-fluoroallylamine hydrochloride; PQR, pyrroloquinoline quinone; BZ, benzylamine; and PLP, pyridoxyl phosphate.

(BSAO), horseradish peroxidase, and semicarbazide were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.); [7-^{14}C]benzylamine was obtained from Amersham (Oakville, Ontario, Canada). Clorgyline [*N*-(2,4-dichlorophenoxy-*n*-propyl)-*N*-methylpropargylamine HCl] was obtained from May & Baker Ltd. (Dagenham, U.K.). (*E*)-2-(4-Fluorophenethyl)-3-fluoroallylamine hydrochloride (MDL-72974A) was a gift from Merrell-Dow Research Institute (Cincinnati, OH). All other chemicals were of analytical grade.

Preparation of SSAOs. Smooth muscle tissues were dissected from dog and bovine aorta as previously described [21]. These smooth muscle tissues along with human umbilical artery and rat aorta tissues were rinsed thoroughly with saline, sliced into small pieces, and homogenized with a Polytron homogenizer (PT-10-35, setting 5 for four periods of 5 sec on ice) in chilled 0.01 M phosphate buffer (pH 6.8). The crude homogenates were centrifuged at 800 *g* for 10 min and the supernatants further centrifuged at 32,000 *g* for 30 min. These final supernatant enzyme preparations were either used immediately or stored at −20°. The enzymes were quite stable under these conditions for at least 1 month.

Preparation of human serum. Blood from healthy subjects was collected with a vacutainer without anticoagulant, kept in a cold room (4°) for 15 min, and centrifuged at 2500 *g* for 10 min. The serum was used for SSAO assay.

Preparation of rat liver mitochondrial MAO. The crude rat liver mitochondrial membrane was used for the estimation of MAO activity. The enzyme was prepared by differential centrifugation as previously described [9].

Determination of SSAO activity. Two methods, radioenzymatic and fluorometric, were used for the estimation of SSAO activities. The radioenzymatic procedure using ^{14}C-labeled benzylamine as substrate followed the procedure previously described for monoamine oxidase assay [9]. The SSAO enzyme preparations were preincubated with clorgyline (1×10^{-4} M) at room temperature for 20 min to ensure that any MAO activity, if present, was completely inactivated. The enzyme was then incubated in the presence of benzylamine (5×10^{-5} M; 0.1 μCi) in a final volume of 200 μL at 37° for 30 min. The enzyme reactions were terminated by adding 200 μL of 2 M citric acid. The oxidized products were extracted into 1 mL toluene:ethyl acetate (1:1, v/v), of which 600 μL was then transferred to a counting vial containing 10 mL. Omnifluor fluid (New England Nuclear, Boston, MA, U.S.A.) Radioactivity was assessed by a liquid scintillation counter (Beckman LS-7500).

The fluorometric procedure was based on the formation of an intense fluorophore formed between homovanillic acid and the hydrogen peroxide released during the oxidation of the amines [15]. The crude SSAO preparations were incubated at 37° for 10 min in the presence of benzylamine in a total volume of 200 μL of 0.05 M phosphate buffer (pH 7.5) containing 50 μg homovanillic acid and 0.82 units of horseradish peroxidase. The developed fluorescence intensity was measured in a spectrophotofluorometer (Aminco-Bowman) at an excitation wavelength of 315 nm and an emission wavelength of 425 nm. The enzyme reactions were linear both with respect to time (for at least 15 min) and to the amount of enzyme used in both measurement procedures.

Protein concentrations were determined by the Bradford method [22] with bovine serum albumin as standard. Kinetic parameters were analyzed according to Wilkinson [23].

RESULTS

Time-dependent inhibition of human umbilical artery SSAO and BSAO. When SSAOs were preincubated with MDL-72974A, a time-dependent inhibition was observed. Figure 1 shows the remaining enzyme activities of human umbilical SSAO and BSAO towards benzylamine following preincubation of the enzyme with MDL-72974A at different concentrations for different time periods. The inhibition was enhanced by prolonging the time of preincubation and by increasing the concentrations of the inhibitor. MDL-72974A inhibition was also found to be more potent towards human umbilical SSAO than towards BSAO.

Irreversible inhibition. Following incubation of SSAOs from the human umbilical artery and rat aorta with MDL-72974A (1×10^{-6} M) for 60 min, the enzyme activities could not be restored either by dialysis or Sephadex G-25 gel filtration (i.e. via a small PD-10 column from Pharmacia) (results not shown). The inactivation of SSAO by MDL-72974A, therefore, is irreversible as is that of MAO [19].

Comparison of the inhibition of MAOs and SSAOs from different sources by MDL-72974A. The effects of MDL-72974A on tissue SSAOs isolated from different species as well as on serum amine oxidases from human and bovine blood were compared with MAO-B and MAO-A isolated from rat mitochondria and human platelet and placenta. As can be seen from panels A and B of Fig. 2, the present study confirmed that MDL-72974A is a highly potent and selective MAO-B inhibitor. The concentration inducing 50% inhibition (IC_{50}) of MAO-B was 5×10^{-10} M with respect to rat liver and human MAO-B and 1×10^{-6} M with respect to MAO-A from both rat liver and human placenta. As can be seen in Fig. 2D, the inhibition of SSAO activities by MDL-72974A varied in different tissues, i.e. IC_{50} values were estimated to be 2×10^{-9} M, 5×10^{-9} M, 8×10^{-8} M and 2×10^{-8} M for SSAO from dog aorta, rat aorta, bovine aorta and human umbilical artery, respectively. MDL-72974A was also quite active towards human serum SSAO, but relatively insensitive towards bovine serum amine oxidases; their IC_{50} values were 2×10^{-8} M and 3×10^{-7} M, respectively (Fig. 2C).

Inhibition kinetics. Figure 3 shows the inhibition of human umbilical SSAO by MDL-72974A with benzylamine as substrate. When the enzyme was preincubated with the inhibitor, the $1/V$ vs $1/S$ values gave straight lines in the absence or presence of MDL-72974A; these lines did not cross at a single point on the X-axis suggesting that the inhibition is

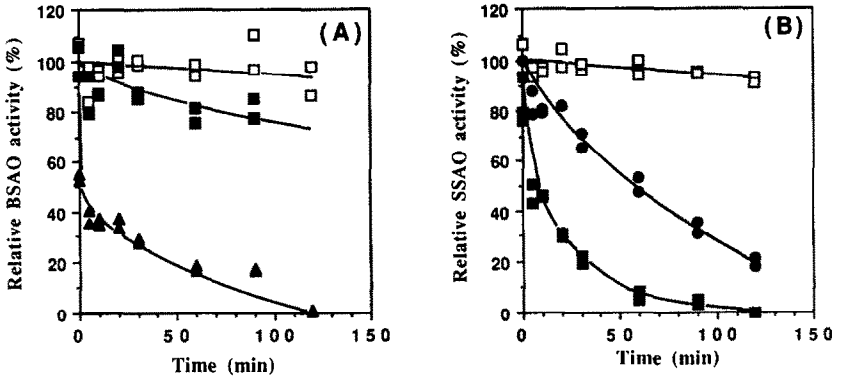

Fig. 1. Time-dependent inhibition of SSAO and bovine serum amine oxidase by MDL-72974A. SSAOs from bovine serum amine oxidase (A) and human umbilical artery (B) were preincubated in the presence or absence of various concentrations of MDL-72974A, i.e. 5×10^{-7} M (▲), 5×10^{-8} M (■), 1×10^{-8} M (●) and no inhibitor (controls) (□) at 20° for different time periods. The remaining enzyme activity was determined by addition of benzylamine as substrate and further incubation at 37° for 10 min.

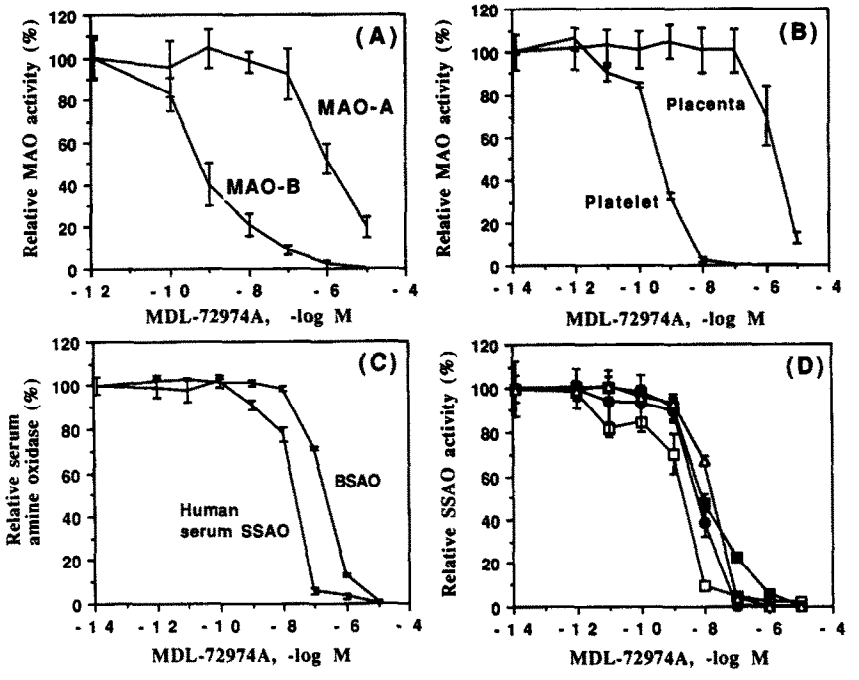

Fig. 2. Effect of MDL-72974A on MAO and SSAO from tissues and sera from different species *in vitro*. MAO-A and MAO-B activities in the rat liver (A), human placenta MAO-A and platelet MAO-B (B), serum amine oxidase in human and bovine sera (C) and tissue SSAOs (D) from human umbilical artery (△) and from dog (□), rat (●) and bovine (■) aortae were assessed. MAO activities towards the B-substrate β-phenylethylamine (5×10^{-5} M) and the A-substrate 5-hydroxytryptamine (5×10^{-4} M) as well as SSAO activities towards benzylamine (5×10^{-4} M) were measured using a radioenzymatic method. Tissue SSAO and serum amine oxidase preparations were preincubated with clorgyline (1×10^{-4} M) to ensure that MAO activities were blocked. The specific activities (nmol/min/mg protein, mean ± SEM) were estimated from three independent experiments. The activities of the untreated control enzymes were: rat liver MAO-B, 7.44 ± 0.63; rat liver MAO-A, 9.23 ± 0.80; human platelet MAO-B, 0.36 ± 0.04; human placenta MAO-A, 5.43 ± 0.55; dog aorta SSAO, 2.79 ± 0.01; rat aorta SSAO, 0.34 ± 0.024; bovine aorta SSAO, 0.26 ± 0.003; human umbilical artery, 2.202 ± 0.016; BSAO, 3.98 ± 0.09; and human plasma amine oxidase, 0.0046 ± 0.0003. The enzymes were preincubated in the absence or presence of different concentrations of MDL-72974A for 20 min at room temperature before addition of substrates.

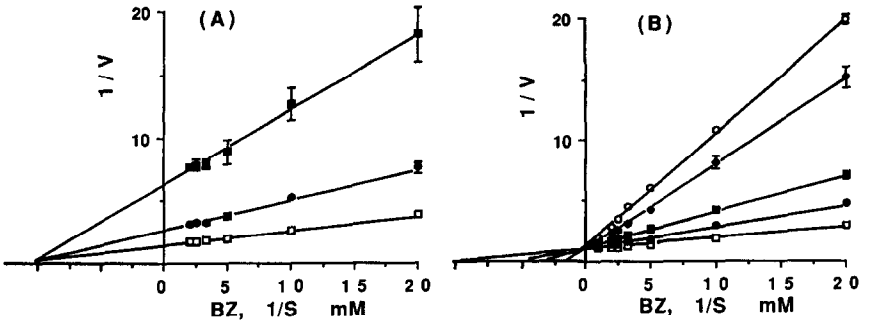

Fig. 3. Lineweaver–Burk plots of the deamination of benzylamine (BZ) catalyzed by human umbilical SSAO activity and the effect of MDL-72974A. The enzyme was preincubated with (A) and without (B) the inhibitor. V = velocity, nmol/mg protein/min; S = substrate concentrations (5×10^{-5} M, 1×10^{-4} M, 2×10^{-4} M, 3×10^{-4} M, 4×10^{-4} M and 5×10^{-4} M. SSAO activity was estimated in the absence (□) and presence of MDL-72974A at 1×10^{-8} M (●), 2×10^{-8} M (■), 4×10^{-8} M (◆) and 8×10^{-8} M (○). Values are means ± SEM of three determinations.

a mixed type of inhibition (Fig. 3A). When the enzyme was not preincubated with the inhibitor, a competitive inhibition was detected (Fig. 3B). The K_m and V_{max} values for the untreated enzyme with respect to benzylamine were estimated to be $1.0 \pm 0.06 \times 10^{-4}$ M and 1.1 ± 0.02 nmol/min/mg protein.

Inhibition of rat aorta SSAO activity in vivo. The effects of the intraperitoneal administration of MDL-72974A on both MAO-A and MAO-B activities in the rat caudate nucleus, cortex and liver and on SSAO activity in the rat aorta were determined. In this study at least two enzyme concentrations for each tissue at different doses of the inhibitor were used for the estimation of the enzyme activities; linear kinetic measurement was thus ensured. As can be seen in Fig. 4, MDL-72974A inhibited both rat brain and liver MAO-B activity selectively. With increasing doses of the inhibitor, MAO-A activity was also affected. Liver MAO-B appeared to be more sensitive to MDL-72974A than that of the brain enzyme under the present experimental conditions. Rat aorta SSAO activity was also found to be strongly blocked by MDL-72974A at low doses.

DISCUSSION

A series of fluoroallylamine derivatives was discovered recently to be irreversible and selective MAO-B inhibitors [24–26]. MDL-72974A is the most potent inhibitor among these compounds inhibiting MAO-B both *in vitro* and *in vivo* [19]. The present study also confirmed that MDL-72974A is an extremely potent MAO-B inhibitor. It was highly selective for the inhibition of MAO-B obtained from rat liver and human platelets with IC_{50} values of about 5×10^{-10} M. It is interesting to note that although MDL-72974A was highly selective for MAO-B, i.e. the A/B ratio with respect to the IC_{50} values was about 1000, it was still a quite potent MAO-A inhibitor, i.e. with IC_{50} values of about 1×10^{-6} M towards rat liver and human placenta MAO-A.

The results presented here indicate that MDL-72974A is also a very potent SSAO inhibitor. This is consistent with previous findings in which several fluoroallylamine MAO-B inhibitors, which are structurally related to MDL-72974A, were capable of inhibiting rat aorta SSAO [27, 28]. The potency of MDL-72974A toward the inhibition of SSAOs varied with respect to species and tissue sources of the SSAO. MDL-72974A-induced inhibition of SSAO was also time dependent. The inhibition was initially competitive, but became non-competitive and irreversible upon incubation. In this study the preincubation time of the enzymes with the inhibitor in each experiment was maintained precisely for 20 min. These analyses, therefore, are not an end point determination. Dog aorta SSAO was found to be the most sensitive to the inhibitor, i.e. with an IC_{50} value of 2×10^{-9} M, which is quite close to that of the inhibition of MAO-B by the same inhibitor. SSAOs from rat aorta, bovine aorta and human umbilical artery were similarly sensitive to MDL-72974A (IC_{50} values in the range of 8×10^{-8} M to 5×10^{-9} M). Bovine serum amine oxidase, however, is relatively insensitive to MDL-72974A. About a 100-fold higher concentration of the inhibitor was required in order to inhibit 50% of the activity of the bovine serum SSAO than was the case for the dog aorta enzyme. Such a variation in sensitivity by different SSAOs towards MDL-72974A supports the notion that SSAO may be quite heterogenous among different species [21].

The inhibition of SSAO by MDL-72974A was competitive, irreversible and time dependent. It appears to be a mechanism-based inhibition following a pseudo-first-order process. Its site of action is not yet known. It has been suggested that (*E*)-(3′,4′-dimethoxyphenyl)-3-fluoroallylamine (MDL-72145), a MAO-B inhibitor with a chemical structure similar to that of MDL-72974A, may be oxidized by MAO to an imine species which then undergoes alkylation by either a nucleophilic enzyme residue or by the flavine cofactor in the active site of the enzyme. Since SSAO does not possess a flavine

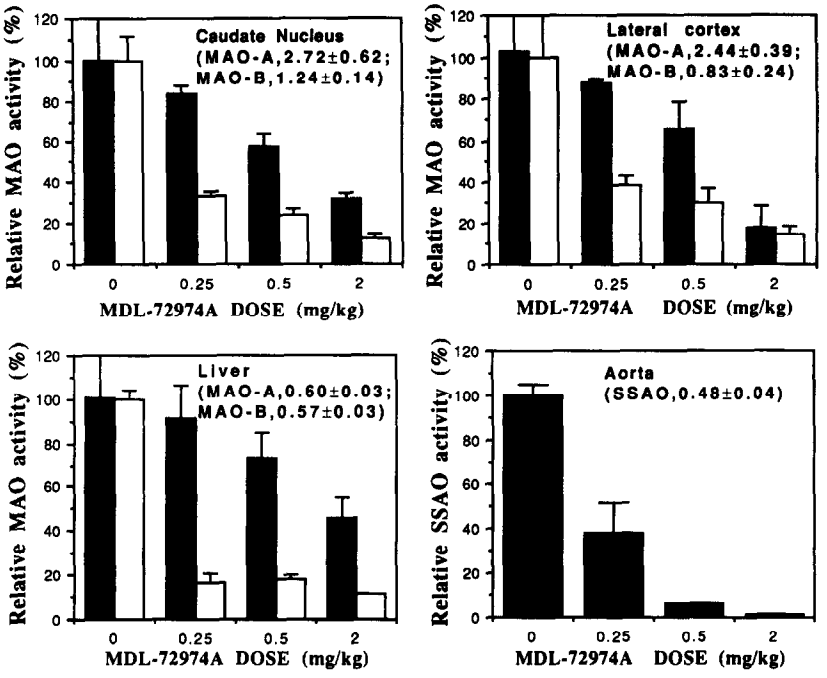

Fig. 4. Effect of MDL-72974A on MAO and SSAO activities *in vivo*. Rats were treated with different doses of MDL-72974A by intraperitoneal injection, and the indicated tissues were dissected 3 hr after the drug administration. The relative MAO activities after drug treatment in the caudate nucleus and lateral cortex, and in the liver towards the B-substrate β-phenylethylamine (5×10^{-5} M) (open bar) and the A-substrate 5-hydroxytryptamine (5×10^{-4} M) (solid bar) as well as SSAO activity in the aorta towards benzylamine (5×10^{-4} M) (shadowed bar) were measured using a radioenzymatic method. The specific enzyme activities (nmol/min/mg protein) of the saline-treated controls in different tissues are indicated in the figure. Values are means ± SEM of three animals at each dose.

moiety, the latter proposal is not possible. The reducing agent dithiothreitol failed to protect against the inhibition of MDL-72974A, thus indicating that a sulfhydryl group from the enzyme is probably not involved.

Although the biochemical properties of bovine serum amine oxidase have been investigated thoroughly, little is known about SSAOs from other species and tissues. These tissue amine oxidases along with BSAO were previously considered to be pyridoxal enzymes containing copper, but this claim was based on the absorption spectra of some of the enzymes [10], and this is a rather crude method. BSAO was then claimed to be a PQQ-containing enzyme [11], but recent work using mass spectrometry and proton nuclear magnetic resonance techniques has indicated that PQQ as a cofactor is also incorrect and that 6-hydroxydopa is the prosthetic group for BSAO [29]. It is still not clear whether or not 6-hydroxydopa is the general prosthetic group for SSAOs from different species, and it is interesting to note that the stereospecificity of the oxidative deamination catalyzed by BSAO is distinctly different from that of rat MAO and rat aorta SSAO [30, 31]. While monoamine oxidase, a flavine enzyme, has been shown to be a stereospecific pro-*R* type of enzyme, rat aorta SSAO is a typical pro-*S* type of enzyme. Bovine serum amine oxidase exhibits no preferential stereospecificity with respect to dopamine or to benzylamine as substrate [30, 31]. The cofactors at the active site could determine the specific configuration and thus determine the stereospecificity between the enzyme and the substrate. The stereospecific structure at the active site of the BSAO may thus be quite different from other SSAOs, such as from rat aorta. The present observation that the BSAO was distinctly less sensitive to MDL-72974A in comparison to other SSAOs further supports this point. The precise chemical nature of the cofactors for different tissue SSAOs remains to be elucidated from purified enzymes.

Following intraperitoneal injection of different doses of MDL-72974A, MAO-B activity in rat brain caudate nucleus, lateral cortex and in rat liver was selectively inhibited. A greater inhibition of MAO-B activity was detected in the liver than in the brain (see Fig. 4). This may well be due to some hindrance such as the blood–brain barrier, which will limit the entry of the drug into the brain during the 3-hr experimental period. Although MDL-72974A appears to be more potent at inhibiting rat liver MAO-B than rat aorta SSAO *in vitro*, it readily inhibits rat aorta SSAO following intraperitoneal injection of the drug at the doses required to produce selective inhibition of MAO-B activity in the rat brain. MDL-72974A can easily penetrate the smooth muscle of the vascular tissues. When MDL-72974A

is applied for the purpose of inhibiting MAO-B activity, i.e. as would be the case when used as an antiparkinsonian drug, it is important to note that SSAO activity will also be affected. The physiological function of SSAO, however, has still not been established, and the pharmacological consequences of SSAO inhibition are unclear.

Acknowledgements—We thank Prof. Alan A. Boulton for his advice and Saskatchewan Health and the Medical Research Council of Canada for their continuing financial support.

REFERENCES

1. Wibo M, Duong AT and Godfraind T, Subcellular location of semicarbazide-sensitive amine oxidase in rat aorta. *Eur J Biochem* **112**: 87–94, 1980.
2. Lewinsohn R, Amine oxidase in human blood vessels and non-vascular smooth muscle. *J Pharm Pharmacol* **33**: 569–575, 1981.
3. Lyles GA and Singh I, Vascular smooth muscle cells: A major source of the semicarbazide-sensitive amine oxidase of the rat aorta. *J Pharm Pharmacol* **37**: 637–643, 1985.
4. Andree TH and Clarke DE, The isolated perfused rat brain preparation in the study of monoamine oxidase and benzylamine oxidase. Lack of selective brain perfusion. *Biochem Pharmacol* **30**: 959–965, 1981.
5. Barrand MA, Callingham BA and Fox SA, Amine oxidase activities in brown adipose tissue of the rat: Identification of semicarbazide-sensitive (clorgyline-resistant) activity at the fat cell membrane. *J Pharm Pharmacol* **36**: 652–658, 1984.
6. Suzuki O and Matsumoto T, Properties of benzylamine oxidase in human small intestine. *Biogenic Amines* **4**: 45–53, 1987.
7. Lyles GA and Bertie KH, Properties of a semicarbazide-sensitive amine oxidase in rat articular cartilage. *Pharmacol Toxicol* **60** (Suppl 1): 33, 1987.
8. Ryder TA, MacKenzie ML, Pryse-Davies J, Glover V, Lewinsohn R and Sandler M, A coupled peroxidatic oxidation technique for the histochemical localization of monoamine oxidase A and B and benzylamine oxidase. *Histochemistry* **62**: 93–100, 1979.
9. Yu PH, Monoamine oxidase. In: *Neuromethods* (Eds. Boulton AA, Baker GB and Yu PH), Vol. 5, pp. 235–272. Humana Press, Clifton, NJ, 1986.
10. Buffoni F, Some contributions to the problem of amine oxidase. *Pharmacol Res Commun* **12**: 101–114, 1980.
11. Lobenstein-Verbeek CL, Jongejan JA, Frank J and Duine JA, Bovine serum amine oxidase: A mammalian enzyme having covalently bound PQQ as prosthetic group. *FEBS Lett* **170**: 305–309, 1984.
12. Clarke DE, Lyles GA and Callingham BA, A comparison of cardiac and vascular clorgyline-resistant amine oxidase and monoamine oxidase. *Biochem Pharmacol* **31**: 27–35, 1982.
13. Precious E, Gunn CE and Lyles GA, Deamination of methylamine by semicarbazide-sensitive amine oxidase in human umbilical artery and rat aorta. *Biochem Pharmacol* **37**: 707–713, 1988.
14. Lyles GA and McDougall SA, The enhanced daily excretion of urinary methylamine in rats treated with semicarbazide or hydralazine may be related to the inhibition of semicarbazide-sensitive amine oxidase activities. *J Pharm Pharmacol* **41**: 97–100, 1989.
15. Yu PH, Oxidative deamination of aliphatic amines by rat aorta semicarbazide-sensitive amine oxidase. *J Pharm Pharmacol* **42**: 882–884, 1990.
16. Yu PH, Deamination of aliphatic amines of different chain lengths by rat liver monoamine oxidase A and B. *J Pharm Pharmacol* **41**: 205–208, 1989.
17. Elliott J, Callingham BA and Sharman DF, Semicarbazide-sensitive amine oxidase (SSAO) of the rat aorta. Interactions with some naturally occurring amines and their structural analogues. *Biochem Pharmacol* **38**: 1507–1515, 1989.
18. Boor PJ and Hysmith RM, Allylamine cardiovascular toxicity. *Toxicology* **44**: 129–144, 1987.
19. Zreika M, Fozard JR, Dudley MW, Bey P, McDonald IA and Palfreyman MG, MDL 72974; A potent and selective enzyme-activated irreversible inhibitor of monoamine oxidase type B with potential for use in Parkinson's disease. *J Neural Transm* [PD-Sect] **1**: 243–254, 1989.
20. Parkinson Study Group, Effect of deprenyl on the progression of disability in early Parkinson's disease. *N Engl J Med* **321**: 1364–1371, 1989.
21. Yu PH, Fang CY and Yang CM, Semicarbazide-sensitive amine oxidase from the smooth muscles of dog aorta and trachea; Activation by the MAO-A inhibitor clorgyline. *J Pharm Pharmacol*, in press.
22. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
23. Wilkinson GN, Statistical estimations in enzyme kinetics. *Biochem J* **80**: 324–332, 1961.
24. McDonald IA, Lacoste JM, Bey P, Palfreyman MG and Zreika M, Enzyme-activated irreversible inhibitors of monoamine oxidase: Phenylallylamine structure–activity relationships. *J Med Chem* **28**: 186–193, 1985.
25. McDonald IA, Palfreyman MG, Zreika M and Bey P, (*Z*)-2-(2,4-Dichlorophenoxy)methyl-3-fluoroallylamine (MDL-72638): A clorgyline analogue with surprising selectivity for monoamine oxidase type B. *Biochem Pharmacol* **35**: 349–351, 1986.
26. Zreika M, McDonald IA, Bey P and Palfreyman MG, MDL-72145, an enzyme-activated irreversible inhibitor with selectivity for monoamine oxidase type B. *J Neurochem* **43**: 448–454, 1984.
27. Lyles GA, Marshall CMS, McDonald IA, Bey P and Palfreyman MG, Inhibition of rat aorta semicarbazide-sensitive amine oxidase by 2-phenyl-3-haloallylamines and related compounds. *Biochem Pharmacol* **36**: 2847–2853, 1987.
28. Elliott J, Callingham BA and Barrand MA, *In-vivo* effect of (*E*)-2-(3′,4′-dimethoxyphenyl)-3-fluoroallylamine (MDL-72145) on amine oxidase activities in the rat. Selective inhibition of semicarbazide-sensitive amine oxidase in vascular and brown adipose tissues. *J Pharm Pharmacol* **41**: 37–41, 1989.
29. Jane SM, Mu D, Wemmer D, Smith JA, Kaur S, Maltby D, Burlingame AL and Klinman JP, A new redox cofactor in eukaryotic enzymes: 6-Hydroxydopa at the active site of bovine serum amine oxidase. *Science* **248**: 981–987, 1990.
30. Yu PH and Davis BA, Stereospecific deamination of benzylamine catalyzed by different amine oxidases. *Int J Biochem* **20**: 1197–1201, 1988.
31. Yu PH, Three types of stereospecificity and the kinetic deuterium isotope effect in the oxidative deamination of dopamine as catalyzed by different amine oxidases. *Biochem Cell Biol* **66**: 853–861, 1988.