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Comparative *ex vivo* inhibitory effects of (*E*)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) on amine oxidase activities in the rat

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Monoamine oxidase (MAO) is a mitochondrial enzyme found in two molecular forms (A and B), having different substrate and inhibitor specificities, and existing in varying proportions in different animal tissues. Recent interest in the therapeutic potential of drugs which act as selective inhibitors of the B-form of MAO, has arisen from favourable clinical experience with L-deprenyl, which is believed to potentiate the benefits of L-dihydroxyphenylalanine (L-DOPA) therapy in Parkinsonian patients by blocking the striatal degradation of dopamine by MAO-B. Also, by sparing MAO-A activity in peripheral tissues, deprenyl administration does not precipitate the occurrence of hypertensive episodes after dietary ingestion of tyramine (reviewed in Ref. 1).

Experimental studies have shown that (*E*)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (MDL 72145) is another selective irreversible inhibitor of MAO-B in rat and mouse brain [2, 3]. Like deprenyl, the administration of MDL 72145 can protect certain laboratory species from the neurodegenerative effects of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) upon the nigrostriatal dopamine pathway (e.g Ref. 4) and *in vitro* MDL 72145 blocks the oxidation of MPTP by MAO-B to putative neurotoxic compounds [5], that are believed to be responsible for the MPTP-induced Parkinsonian-like state which can occur in man. Thus, MDL 72145 is another potential anti-Parkinsonian agent with MAO-B inhibitory properties, without however, the additional amphetamine-like actions exhibited by deprenyl and its metabolites in rodents [3, 6].

We showed recently that MDL 72145 and some related analogues are also potent irreversible inhibitors *in vitro* of a semicarbazide-sensitive amine oxidase (SSAO) in the rat aorta [7, 8]. This enzyme is particularly active in vascular smooth muscle cells [9], probably as a plasmalemmal component [10], and it is distinguishable from MAO activities

by its resistance to acetylenic MAO inhibitors such as clorgyline and deprenyl, and by its sensitivity to semicarbazide and related compounds (reviewed in Ref. 11). Although SSAO is especially active against the synthetic substrate benzylamine, the nature of its physiological substrate(s) and its importance in mammalian degradation of endogenous amines remains to be determined (see Discussion). The emergence of new inhibitors of SSAO to use in addition to established compounds for investigating the enzyme's properties and function may therefore be of some value. For this reason, we have determined if significant inhibition of SSAO activity occurs in rats after administration of MDL 72145 in doses previously used to study its relative effects on MAO-A and B. Preliminary details of this study have been reported earlier in abstract form [12].

Materials and methods

Animals and chemicals. Male Sprague–Dawley rats (140–160 g) were obtained from our Departmental Breeding Colony, Animal Services Unit, University of Dundee. (7-^{14}C)-benzylamine hydrochloride and 5-hydroxy-(G-^{3}H)-tryptamine (5-HT) creatinine sulphate were purchased from Amersham International (Amersham, U.K.). The hydrochloride salt of MDL 72145 was a gift from Dr M. G. Palfreyman (Merrell-Dow Research Institute, Cincinnati, U.S.A.).

Drug administration and enzyme assays. Rats were divided into five groups, containing five or six animals, and treated by intraperitoneal (i.p.) injection with MDL 72145 hydrochloride dissolved in saline (aqueous 0.9% NaCl, w/v) vehicle at the following doses: 0 (controls), 0.05, 0.1, 1 and 10 mg/kg. Control animals received corresponding volumes (5 ml/kg) of vehicle alone. Rats were killed 24 hr later and various tissues removed from each animal for storage at −20°, before use in enzyme assays within two weeks. All samples of a given tissue type were subsequently homogenized and assayed at the same time.

Thawed tissues were homogenized in 1 mM potassium phosphate buffer pH 7.8 at tissue (g): buffer (ml) ratios of 1:5 (brain), 1:10 (liver, lung and heart) and 1:40 (aorta). After centrifugation of homogenates (600 *g*/10 min), the resulting supernatants were used as the source of most enzyme activities determined, but samples of aorta and lung supernatant were further diluted six- and ten-fold, respectively, for assay of SSAO.

General details of the radiochemical assays employed are given elsewhere (e.g. Ref. 13). Substrates used here were 1 mM 5-HT (sp. act. 2 μCi/μmole) for MAO-A activity, 1 mM benzylamine (0.5 μCi/μmol) for MAO-B and 1 μM benzylamine (10 μCi/μmole) for SSAO. Various reports have previously established the selectivity of these substrates for the appropriate enzymes in the tissues examined here [11, 14, 15].

Protein concentrations of homogenates were determined by the method of Lowry *et al.* [16].

Results and discussion

Figure 1 shows the dose-related inhibition of MAO-A and B by MDL 72145 in the rat brain and liver under the *ex vivo* conditions employed here. Estimated inhibitor doses (ED_{50}, mg/kg) to produce 50% inhibition of enzyme activities were 2.2 (brain) and 4.0 (liver) for MAO-A, and 0.30 (brain) and 0.45 (liver) for MAO-B. These results agree broadly with earlier reports identifying doses of MDL 72145 which produce selective inhibition of MAO-B in the brain of the mouse (after i.p. injection) and the rat (after oral administration) [2, 3], although in the current study with i.p. treatment of rats, the range of inhibitor doses showing this relative selectivity appeared to be rather narrower, with only 1 mg/kg demonstrating this clearly in the brain. In the liver, a tissue not included in earlier reports, MDL 72145 was relatively more selective for MAO-B at 0.1 and 1 mg/kg.

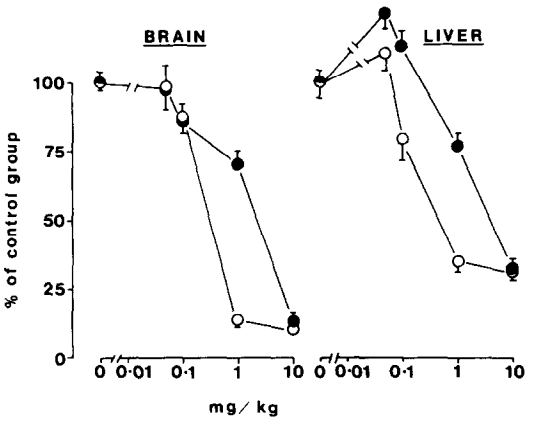

Fig. 1. *Ex vivo* inhibition of MAO-A and B by various doses of MDL 72145. Rats were killed 24 hr after i.p. drug administration. MAO-A (●) and MAO-B (○) activities were assayed with 1 mM 5-HT and benzylamine, respectively. Mean values (±SE) in drug-treated groups (each containing five animals) were expressed as percentages of the corresponding control (saline-treated) group, which contained six rats. Control specific activities (nmoles/hr/mg protein) were for MAO-A: 19.4 ± 0.6 (brain), 32.9 ± 1.5 (liver), and for MAO-B: 39.2 ± 1.1 (brain), 117.2 ± 6.6 (liver).

Figure 2 compares the effects of MDL 72145 upon MAO-A and SSAO activity in heart, aorta and lung. Estimated ED_{50} doses (mg/kg) were 3.5 (heart), 8.9 (aorta), 4.2 (lung) for MAO-A and 0.60 (heart), 0.33 (aorta), 0.40 (lung) for SSAO. Thus, overall, MDL 72145 exhibits a potency as an inhibitor of SSAO in these tissues similar to that as an inhibitor of MAO-B in brain and liver. These results show, therefore, that the use of the drug as a research tool to achieve selective MAO-B inhibition *in vivo* in rats will also produce substantial inhibition of SSAO activity in various tissues, a property which should be kept in mind when investigating the pharmacological effects of the compound.

In all of the tissues examined here, the inhibition of MAO-B and SSAO produced by 10 mg/kg MDL 72145 was similar to that found with 1 mg/kg. In the rat brain, almost complete inhibition of MAO-B occurred after these doses. However, elsewhere, a plateau level of around 60–75% inhibition of MAO-B or SSAO resulted. In this study, tissues were removed 24 hr after drug administration to the animals, a similar protocol to that used by others [2, 3]. It is possible that some recovery from greater levels of irreversible inhibition may occur by the process of *de novo* synthesis of enzyme activities during this time, especially if residual free inhibitor drug were to be cleared from the animal relatively quickly. The recovery process would be more rapid for MAO in tissues such as the liver and heart (in young rats) where half-lives for enzyme recovery are relatively short (around 3–6 days) [17, 18] compared with the rat brain (10–13 days) [19]. Information on the turnover of SSAO is limited, although half-lives of around 5–6 days in rat heart and aorta after inhibition by benserazide have been reported [20]. Such factors governing enzyme recovery rates may account for the lack of more complete inhibition of enzyme activities by the higher doses of MDL 72145 in some of the tissues studied here.

The physiological consequences of inhibiting SSAO *in vivo* are not yet obvious. Kinetic constants determined for the metabolism of tyramine, tryptamine and β-phenylethylamine (PEA) in vascular homogenates have indicated

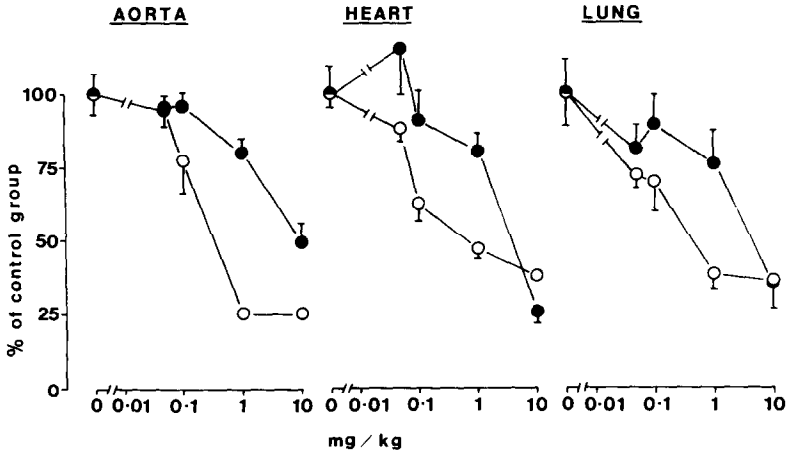

Fig. 2. *Ex vivo* inhibition of MAO-A and SSAO by various doses of MDL 72145. Rats were killed 24 hr after i.p. drug administration. MAO-A (●) and SSAO (○) activities were assayed with 1 mM 5-HT and 1 μM benzylamine, respectively. Mean values (±SE) in drug-treated groups (each containing five animals) were expressed as percentages of the corresponding control (saline-treated) group, which contained six rats. Control specific activities (nmoles/hr/mg protein) were for MAO-A: 19.2 ± 1.4 (aorta), 60.7 ± 5.9 (heart), 8.3 ± 1.0 (lung) and for SSAO: 23.3 ± 1.5 (aorta), 0.66 ± 0.03 (heart), 2.5 ± 0.3 (lung).

that SSAO may contribute, with MAO, to the endogenous degradation of these amines in the rat [21, 22]. To support this, Guffroy *et al.* [21] found that plasma concentrations of [^{14}C]PEA reached greater peak levels, and declined more slowly, after intravenous administration of the labelled amine to semicarbazide-pretreated rats, compared with control animals. We have shown recently that the aliphatic amine methylamine, which is not metabolized by MAO activities, is also a good substrate for SSAO in rat aorta homogenates [13], and furthermore, a 3- to 6-fold increase in daily urinary excretion of methylamine was found in rats after treatment with SSAO inhibitors [23]. Organ bath studies of the possible functional consequences of SSAO inhibition have identified experimental conditions under which the contractile effects of PEA, tryptamine and certain other trace amines may be potentiated in rat vascular and non-vascular smooth muscle preparations after exposing tissues to SSAO inhibitors [24, 25].

The use of rat tissues in such studies may have restricted value in predicting the likely effects of SSAO inhibition in man. The human vascular enzyme differs from that in the rat by having a much lower affinity for benzylamine, in addition to displaying extremely poor degradative activity towards those aromatic trace amines above (e.g. Refs 13, 26). However, the human enzyme has good activity against methylamine, suggesting that SSAO might be involved in aliphatic amine metabolism in man [13, 26]. Determinations of plasma or urinary methylamine concentrations in patients treated with drugs which have the incidental property of inhibiting SSAO [11] may help to clarify this issue.

In conclusion, therefore, we have demonstrated that the *ex vivo* potency of MDL 72145 as an inhibitor of SSAO in the rat is similar to its potency against MAO-B, a result consistent with previous *in vitro* comparisons [7, 8]. However, since SSAO shows species-related differences in its ability to bind and metabolize amine substrates, it is possible that the sensitivity of this enzyme to inhibition by drugs such as MDL 72145 and other compounds which are substrate analogues, may also vary between species. This should be borne in mind when the possible effects of SSAO inhibitors in man are under investigation.

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