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Inhibition of semicarbazide-sensitive amine oxidase by monoamine oxidase B inhibitors from the oxazolidinone series

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The purpose of the present work was to study the semicarbazide-sensitive amine oxidase (SSAO) inhibitory properties of MD 240931 and MD 240928 (the two enantiomers of MD 780236) as well as those of the corresponding primary amines, MD220662 and MD220661, in rat heart and aorta. MD 240928 and MD 240931 are rather weak SSAO inhibitors, MD 240931 being more potent than MD 240928. Of the four compounds studied, the most potent inhibitor of SSAO is MD 220662, its IC50 value ranging from $2 \cdot 10^{-6}$ to $6 \cdot 10^{-6}$ M. The SSAO inhibitory potency of this compound does not change significantly with the time of preincubation in both the absence and presence of clorgyline (10^{-4} M). MD 220661 is also an inhibitor of SSAO; however, its SSAO inhibitory potency, which without preincubation is comparable to that of MD 220662, does decrease with the time of preincubation to the same extent in both the absence and presence of clorgyline (10^{-4} M). These results suggest that MD 220661 is not only an inhibitor of SSAO, but is also a substrate of the enzyme.

MD 780236 is a selective inhibitor of the B form of the enzyme monoamine oxidase (MAO; EC 1.4.3.4.) in rat tissues (Strolin Benedetti et al 1982; Tipton et al 1983).

The different stereoselective inhibition of MAO-B by the *R* (MD 240928) and *S* (MD 240931)-enantiomers of MD 780236 has been described (Dostert et al 1983; Tipton et al in the press), as well as the metabolism of MD 780236 and its enantiomers by the A and B forms of the enzyme in the rat (Strolin Benedetti & Dow 1983; Strolin Benedetti et al 1983). Moreover the metabolism of MD 240928 through oxidative deamination has been demonstrated not only in rat (Strolin Benedetti et al in press; Strolin Benedetti et al 1984) but also in dog and man (Strolin Benedetti et al in the press).

Recently, the effect of these compounds on the semicarbazide-sensitive amine oxidase (SSAO) has been described: according to Callingham et al (in the press) these compounds possess some inhibitory properties of SSAO from rat brown adipose tissue, the K_i values being 8·1, 4·2 and 20×10^{-5} M for MD 780236, MD 240931 and MD 240928 respectively. Inhibition of this enzyme in rat testis and lung by MD 780236 has been studied by Kinemuchi et al (in the press), who have reported an IC50 value of 17×10^{-5} M.

The purpose of the present work was to study the SSAO inhibitory properties of MD 240931 and MD 240928 as well as those of the corresponding primary amines (MD 220662 and MD 220661), which are also MAO-B inhibitors (Strolin Benedetti et al in the press; Strolin Benedetti et al 1984), in rat heart and aorta. It would be useful to have this information for the primary amines because MD 220661, which was found to be an important metabolite of MD 240928 in rat brain, is also a reversible and selective inhibitor of MAO-B as the parent compound (Strolin Benedetti et al 1984). The reason for choosing aorta was that this tissue has an important SSAO activity as it has a great capacity to deaminate 1 μM benzylamine (Guffroy & Strolin Benedetti 1984). The reason for choosing heart was that SSAO inhibitors seem to protect against the cardiotoxicity of some exogenous amines (Nelson & Boor 1982).

Materials and methods

The compounds studied have been synthesized in the Department of Organic Chemistry of the Delalande Research Centre. Their structural formulae as well as their respective code numbers are presented in Fig. 1. All the compounds were prepared as methane sulfonate salts.

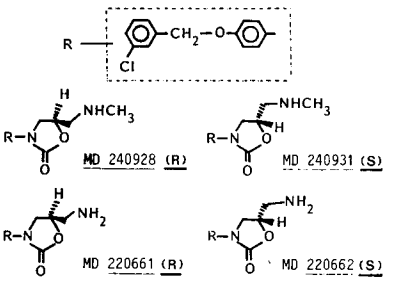

FIG. 1. Structural formulae of the compounds studied.

Clorgyline hydrochloride was also synthesized in the Department of Organic Chemistry of the Delalande Research Centre whereas semicarbazide hydrochloride was obtained from Merck, Darmstadt, F.R. Germany.

Male Wistar rats (Iffa Credo, L'Arbresle, France) 260–300 g, were used. Heart and thoracic aorta were removed, rinsed in 0·9% NaCl (saline), and after adhering tissue was dissected away, blotted dry, weighed, frozen in liquid nitrogen and stocked at −20 °C until used.

Heart and aorta MAO and SSAO activities were measured as described by Guffroy & Strolin Benedetti (1984), using 100 μM 5-hydroxytryptamine (5-HT) and 1 or 12 μM benzylamine as substrates. Previous experi-

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ments (Guffroy & Strolin Benedetti 1984) have shown that deamination of 100 μM 5-HT is carried out exclusively by MAO-A in heart and aorta and that deamination of 1 and 12 μM benzylamine is carried out exclusively by SSAO in heart and aorta respectively. The MAO and SSAO inhibitory properties of the compounds have been studied following different pre-incubation times and in presence or absence of selective MAO-A and SSAO inhibitors, clorgyline (1×10^{-4} M) and semicarbazide (1×10^{-3} M) respectively, (60 and 30 min respectively, before pre-incubation with the studied compounds). Protein concentrations of the homogenates were determined by the method of Lowry et al (1951).

Preliminary ex-vivo experiments have been carried out with MD 220661 and MD 220662 after i.v. injection in the rat. The compounds have been administered at doses of 10, 25 and 50 mg kg^{-1} (dose expressed as base), the rats killed 15 min after dosing and inhibition of SSAO in the heart homogenate (1 g/20 ml sodium phosphate buffer, pH 7·8) measured using 1 μM benzylamine.

The IC50 values were determined on a Tektronix 4052 computer according to the logistic model of De Lean et al (1978). For time-course and dose-dependent inhibition, statistical analyses were performed on the experimental data using the Dunnett's test (Dunnett 1955) with validation of homogeneous variance by the Bartlett's test (Armitage 1973).

Results and discussion

The IC50 values towards SSAO for the four compounds studied are given in Table 1. MD 240928 and MD 240931 show some SSAO inhibitory properties in aorta and heart, confirming the results reported by Callingham et al on other rat tissues; the two compounds are rather weak SSAO inhibitors, MD 240931 being more potent than MD 240928.

Of the four compounds studied, the most potent inhibitor of SSAO is MD 220662, its IC50 value ranging from $2 \cdot 10^{-6}$ to $5 \cdot 10^{-6}$ M and from $3 \cdot 10^{-6}$ to $6 \cdot 10^{-6}$ M in aorta and heart respectively. The SSAO inhibitory potency of this compound does not change significantly with the time of preincubation (Fig. 2a) in both the absence and presence of clorgyline (10^{-4} M).

MD 220661 is also an inhibitor of SSAO, its IC50 value ranging from 3×10^{-6} to 3×10^{-5} M and from 2×10^{-6} to 5×10^{-6} M in aorta and heart respectively (Table 1). However, the SSAO inhibitory potency of this compound, which without preincubation is comparable to that of MD 220662, does decrease with the time of preincubation (Fig. 3a), to the same extent in both the absence and presence of clorgyline (10^{-4} M).

MD 220662 and MD 220661 are also inhibitors of MAO-A in the two tissues studied; the MAO-A

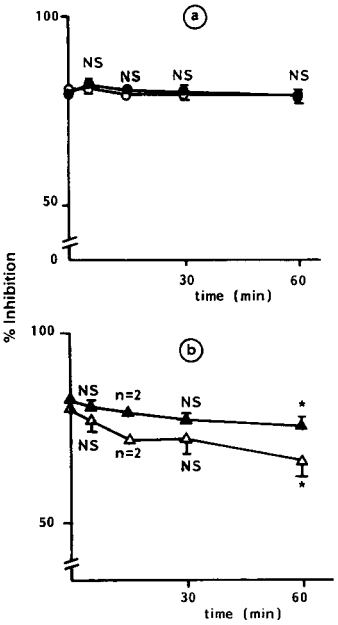

FIG. 2. Time-course of inhibition of SSAO and MAO-A activity in rat aorta by MD 220662 at a concentration of 1×10^{-5} M. Mean values ± s.d. of 3–4 homogenates pooled from 3 organs. Substrates used were: (a) benzylamine 12 μM with (●) and without (○) clorgyline 1×10^{-4} M, (b) 5-HT 100 μM with (▲) and without (△) semicarbazide 1×10^{-3} M. Dunnett test: NS = not significant, $^*P < 0{\cdot}05$.

Table 1. Inhibition of rat aorta and heart SSAO by MD 240928, MD 240931, MD 220661 and MD 220662. All values are the mean ± s.d. of 3 homogenates pooled from 3 organs.

	IC50 (μM) in aorta (benzylamine 12 μM) Proteins: 0·4 mg ml^{-1} Incubation time: 2 min				IC50 (μM) in heart (benzylamine 1 μM) Proteins: 1·6 mg ml^{-1} Incubation time: 5 min			
	without clorgyline		with clorgyline 1×10^{-4} M 1 h, 37 °C		without clorgyline		with clorgyline 1×10^{-4} M 1 h, 37 °C	
Preincubation time (min) with compound	0	60	0	60	0	60	0	60
MD 240928	>100	>100	>100	>100	>100	>100	>100	>100
MD 240931	16·2 ± 0·4	24·0 ± 0·5	19·4 ± 0·6	32·5 ± 2·2	15·9 ± 1·5	15·6 ± 0·6	23·5 ± 2·8	26·2 ± 5·6
MD 220661	3·0 ± 0·3	32·7 ± 2·0	4·1 ± 0·7	27·1 ± 5·0	1·9 ± 0·1	5·4 ± 0·7	2·4 ± 0·2	7·1 ± 1·0
MD 220662	2·0 ± 0·2	4·9 ± 0·7	1·8 ± 0·1	1·8 ± 0·3	3·0 ± 0·4	6·3 ± 0·8	3·0 ± 0·2	4·9 ± 1·2

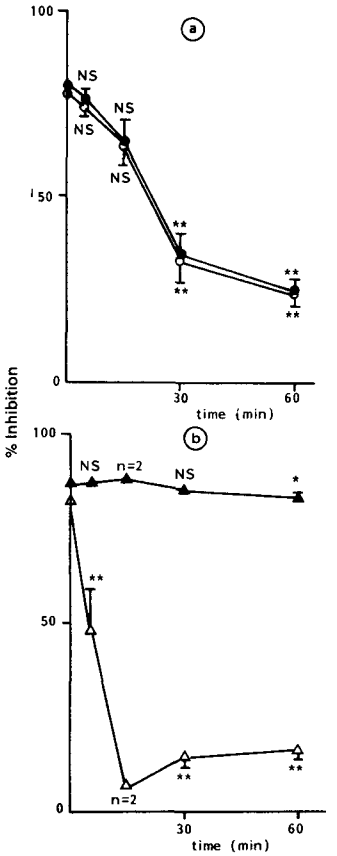

FIG. 3. Time-course of inhibition of SSAO and MAO-A activity in rat aorta by MD 220661 at a concentration of 1×10^{-5} M. Mean values ± s.d. of 3–4 homogenates pooled from 3 organs. Substrates used were: (a) benzylamine 12 μM with (●) and without (○) clorgyline 1×10^{-4} M, (b) 5-HT 100 μM with (▲) and without (△) semicarbazide 1×10^{-3} M. Dunnett test: NS = not significant, $*P < 0.05$, $**P < 0.01$.

inhibitory potency of MD 220661 in aorta decreases as a function of preincubation time (Fig. 3b) in the absence of semicarbazide, but diminishes only slightly in the presence of 10^{-3} M semicarbazide; the MAO-A inhibitory potency of MD 220662 in aorta is only slightly decreased by preincubation, in both the absence and presence of semicarbazide (Fig. 2b).

These results suggest that MD 220661 is not only an inhibitor of SSAO, but is also a substrate of the enzyme, whereas MD 220662 is an inhibitor of SSAO and might be a poor substrate of the enzyme, if at all.

As mentioned above, the percentage inhibition of 5-HT deamination by MD 220661 and MD 220662 in aorta in the presence of semicarbazide diminishes only very slightly as a function of preincubation time; moreover the percentage inhibition of 10 μM phenylethylamine deamination by MD 220661 in rat liver mitochondria in absence of clorgyline does not diminish as a function of preincubation time (Boucher & Strolin Benedetti, unpublished results). This is in contrast to the observation with the corresponding secondary amine MD 240928 (Tipton et al in the press), which is a substrate of MAO-A, as is the other enantiomer MD 240931 (Tipton et al in the press; Strolin Benedetti et al 1983).

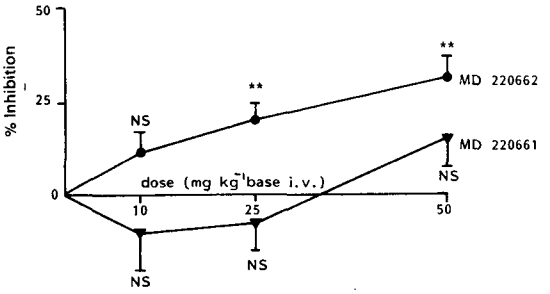

FIG. 4. Ex-vivo inhibition of SSAO activity in rat heart by MD 220661 and MD 220662 at different doses. The compounds were injected i.v. in NaCl 0·9% and the rats killed 15 min after. SSAO activity was tested with benzylamine 1 μM. Mean values ± s.e.r. from 5 animals. Dunnett test: NS = not significant, $*P < 0.05$, $**P < 0.01$.

The results of the preliminary ex-vivo experiments show that (Fig. 4) a significant although weak inhibition of heart SSAO activity results from the dose of 25 mg kg^{-1} after injection of MD 220662, whereas no significant inhibition is observed after injection of MD 220661 even at 50 mg kg^{-1}. This supports the fact that the latter compound, being a substrate of SSAO, is rapidly metabolized by the enzyme in-vivo.

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α-Adrenoceptor blocking action of aaptamine, a novel marine natural product, in vascular smooth muscle

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In the rabbit isolated aorta and renal artery, aaptamine (3×10^{-5} M), a novel heteroaromatic substance isolated from a sea sponge *Aaptos aaptos* produced a parallel, rightward shift of the dose-response curve for noradrenaline, whereas that for histamine or KCl was not affected. But, the derivatives of aaptamine, demethylaaptamine, demethyloxyaaptamine, dihydroaaptamine and dihydrodemethylaaptamine at concentrations of 10^{-5} to 10^{-4} M had no effect on the dose-response curve for noradrenaline. These results suggest that aaptamine is a competitive antagonist of α-adrenoceptors in vascular smooth muscles.

A number of natural products isolated from marine organisms have proved to very useful chemical tools for pharmacological, physiological and biochemical studies as they have been shown to act on specific sites of the cell membrane (Narahashi 1974; Catterall 1980; Ohizumi et al 1983; Takahashi et al 1983). In the course of our survey on pharmacologically active substances in marine organisms, much attention has been given to the occurrence of natural products possessing an α-adrenoceptor blocking activity, since these substances have played an important role in basic and clinical pharmacology (Gross 1980). Recently, a sea sponge *Aaptos aaptos* has been revealed to have a marked α-adrenoceptor blocking activity in the rabbit isolated aorta. From this sponge heteroaromatic compound named aaptamine, with a novel skeleton, 1H-benzo[de]-1,6-naphthyridine, has been isolated as an active substance (Nakamura et al 1982). The present study was undertaken to characterize the pharmacological properties of aaptamine and its derivatives using vascular smooth muscle.

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Methods

Male albino rabbits (2–3 kg) were killed by a blow on the head. The thoracic aorta and the renal artery were excised and suspended in a Krebs-Ringer-bicarbonate solution of the following composition (mM): NaCl, 120; KCl, 4·8; $CaCl_2$, 1·2; $MgSO_4$, 1·3; KH_2PO_4, 1·2; $NaHCO_3$, 25·2 and glucose, 5·8, at pH 7·4 and were aerated with a gas mixture of 95% O_2 and 5% CO_2. The method of preparing the aorta was as described by Ohizumi & Yasumoto (1983). After the connective tissue had been removed, the renal artery was cut into helical strips approximately 1 mm wide and 4 mm long. A resting tension of 1 g was applied to each strip and isometric contractions were measured by a force-displacement transducer and recorded on a polygraph.

The following drugs were used: noradrenaline bitartrate (Sigma) and histamine dihydrochloride (Wako Pure Chemical). Aaptamine hydrochloride, demethylaaptamine hydrochloride and demethyloxyaaptamine were isolated from the sea sponge *Aaptos aaptos* collected at Okinawa island and dihydroaaptamine hydrochloride and dihydrodemethylaaptamine hydrochloride were prepared as described by Nakamura et al (1982, 1983). Dihydroaaptamine hydrochloride or demethyloxyaaptamine was dissolved in ethanol to a final concentration of 0·1%. All other drugs were dissolved in distilled water as required.

Results and discussion

In both the aorta and the renal artery, NA (10^{-8} to 3×10^{-6} M), histamine (10^{-6} to 10^{-4} M) or KCl (10^{-2} to 4×10^{-2} M) caused a concentration-dependent contraction. As shown in Fig. 1, after treatment of the aorta with