J Neural Transm (2002) 109: 251–265

Journal of Neural Transmission

Inhibition of monoamine oxidase modulates the behaviour of semicarbazide-sensitive amine oxidase (SSAO)

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Received November 22, 2001; accepted December 21, 2001

Summary. The specific activity and kinetic behaviour of semicarbazide-sensitive amine oxidase (EC 1.4.3.6; SSAO) towards benzylamine, in the rat heart, is affected by in vivo treatment with the non-selective monoamine oxidase (MAO) inhibitor tranylcypromine, but not by the selective MAO-A and -B inhibitors, clorgyline and lazabemide. SSAO activity was increased to 178% of control activity after 7 days of treatment with tranylcypromine. This increase appears to represent an increase in the limiting velocity (V_{max}) for benzylamine oxidation with no significant change in the K_m at that time-point. However, the K_m for benzylamine oxidation was found to decrease in both controls and treated groups, in a time-dependent manner, during the treatment regime. These findings suggest a link between SSAO and cellular stress and may have importance in the context of the recent finding that tissue-SSAO is identical to a vascular adhesion protein (VAP1), involved in the process of inflammation.

Keywords: Tranylcypromine, lazabemide, clorgyline, dopamine.

Abbreviations

DHBA 3,4-dihydroxybenzylamine, *L-DOPA* 3,4-dihydroxy-L-phenylalanine, *DOPAC* 3,4-dihydroxyphenylacetic acid, *5-HIAA* 5-hydroxyindole acetic acid, *HVA* homovanillic acid, *MAO* monoamine oxidase, *PCA* perchloric acid, *PEA* 2-phenylethylamine, *SSAO* semicarbazide-sensitive amine oxidase, *TTBS* tween tris-buffered saline, *VAP* vascular-adhesion protein.

Introduction

The monoamine oxidase (MAO) enzymes (EC. 1.4.3.4.) catalyse the oxidative deamination of biologically-active amines in the central nervous system, where their actions modulate neurotransmitter activity, and in the periphery, where regulation of the amine levels by amine oxidases and other enzymes,

such as catechol-O-methyl transferase, facilitate the processes of detoxification and vaso-modulation (see Youdim and Finberg, 1991 for review). Another group of amine oxidases, distinct from classical MAO-A and MAO-B, can also catalyse the oxidative deamination of many of the substrates more commonly associated with MAO, such as 2-phenylethylamine (PEA), benzylamine, tyramine, dopamine and, in some tissues, 5-hydroxytryptamine (5-HT), as well as some compounds which are not substrates for MAO, such as aminoacetone and methylamine (see Lyles et al., 1995a,b for reviews). Unlike the classical monoamine oxidase activities, this enzyme group is insensitive to inhibition by acetylenic MAO inhibitors, such as clorgyline (Clarke et al., 1982). It is inhibited by carbonyl-group reagents such as semicarbazide and is frequently referred to as semicarbazide-sensitive amine oxidase (SSAO, EC.1.4.3.6). Its activity, which is high in cardiovascular tissue, is elevated in diabetes, congestive heart failure and following severe burns (Lewinsohn, 1977). It is also increased in brown adipose tissue of obese Zucker rats (Barrand and Callingham, 1982).

A link has recently been reported between the glucose transporter GLUT 4 and SSAO activity that may have important implications for SSAO in non-insulin dependent diabetes and other disorders involving glucose transport (see Enrique-Tarancon et al., 1998). Soluble SSAO has recently been found to be identical to vascular-adhesion protein 1 (VAP1), a cell adhesion molecule involved in the binding of lymphocytes to the endothelium under inflammatory conditions (see Jalkanen and Salmi, 2001). SSAO activity at sites of inflammation, may function to reduce the local concentration of amines.

It has been suggested that SSAO may compensate, at least to some extent, for the deficit in the oxidative deaminating capacity in situations where MAO activity is dysfunctional (Murphy et al., 1991). Such a situation might arise from the therapeutic use of monoamine oxidase inhibitors. The MAO-B inhibitor l-deprenyl (selegiline) is widely used in the treatment of that disease and may also be of value in Alzheimer's disease, whereas MAO-A inhibitors and non-selective MAO inhibitors have been widely used as antidepressants. The ability of SSAO to catalyse the oxidative deamination of at least some of the excess amines following such treatments may thus become important in these conditions. Furthermore, the use of L-dihydroxyphenylalanine (L-DOPA) in the treatment of Parkinson's disease may result in an increased flux through SSAO as the dopamine generated overloads MAO (Lizcano et al., 1991).

The activity of MAO A and B is also reduced in the atypical form of Norrie disease, in which patients lack the genes encoding either one or both isoforms of MAO (Warburg, 1966; de la Chapelle et al., 1985). Murphy et al. (1990) reported the urinary levels of the biologically active amines, PEA, o, m- and p-tyramine and noradrenaline and their metabolites, to be severely affected in two subjects suffering from the atypical form of Norrie disease, whereas the concentrations of the metabolites of dopamine and 5-HT were not significantly altered. Similar biochemical abnormalities in the levels of catecholamines and their metabolites in Norrie diseased individuals have been reported by Lenders et al. (1996). The amine and amine metabolite

levels in depressed individuals receiving MAO inhibitor treatment has also shown some similarity to the situation in Norrie disease (Murphy et al., 1981; Karoum et al., 1982).

The alterations in the metabolism of PEA, tyramine and the shift towards the O-methylation pathway of noradrenaline found in atypical Norrie disease are consistent with the absence of functional MAO-A and -B activities. However, the finding that the levels of the deaminated metabolites of dopamine (dihydroxyphenylacetic acid; DOPAC, and homovanillic acid; HVA) and of dopamine itself were not significantly altered in these subjects, is difficult to reconcile with this. The activity of SSAO towards dopamine (Mc Ewen et al., 1965; Lizcano et al., 1991) and the fact that the genes for SSAO do not appear to be associated with the MAO-A and -B loci (Zhang and McIntire, 1996; Ozelius et al., 1988) suggest that it might contribute to the metabolism of this amine in atypical Norrie disease. Furthermore, it has been reported that the levels of human plasma SSAO increase in response to the infusion of dopamine (Zis et al., 1981). This response was rapid and accompanied by a disappearance of plasma dopamine, despite a constant continuous rate of infusion of that amine. Although SSAO may also metabolise MAO substrates in individuals treated with some MAO inhibitors, such effects may not operate in the CNS, since SSAO is absent from brain tissue. However, cardiovascular SSAO activity is present in the brain microvessels (Zuo and Yu, 1994), where modulation of its activity might play a role in protecting the brain from elevated concentrations of circulating amines. Data obtained in vivo with the selective MAO-B inhibitors deprenyl and pargyline are, however, difficult to interpret, since these compounds may be metabolised to propargylamine derivatives that inhibit SSAO (Callingham et al., 1985).

In order to study the possibility that SSAO may compensate for loss of MAO activity, the effects of long-term inhibition of MAO, on the levels and kinetic behaviour of SSAO in the rat, were studied. Tranylcypromine was chosen as it is an irreversible, non-selective inhibitor of MAO (see e.g. Reynolds et al., 1980) that does not inhibit SSAO at the in vivo concentrations required to inhibit MAO. Clorgyline was chosen as a selective inhibitor of MAO-A and lazabemide as a selective inhibitor of MAO-B, as these compounds have not been reported to inhibit SSAO activity in vivo. The effect of MAO inhibition on the metabolism of dopamine and 5-HT in the striatum was studied to show whether SSAO might oxidise these amines under these conditions.

Materials and methods

[^{14}C]-Benzylamine HCl, was obtained from Amersham International and [^{14}C]-tyramine HCl was obtained from New England Nuclear (N.E.N.). Chemicals were diluted with the corresponding unlabelled substrates (Sigma Chemical Co.). HPLC-grade methanol was from BDH Chemicals. "Vectastain" ABC Kit, for visualising Western blots, was obtained from Vector Laboratories, Peterborough. Anti-bovine lung SSAO antibodies were a kind gift from Drs Mercedes Unzeta and Jose-Miguel Lizcano, Department of Biochemistry and Molecular Biology, Autonomous University of Barcelona. Lazabemide (Ro-19-6327) was a kind gift from Dr. Grayson Richards, Hoffman-la Roche, Basel, Switzerland. All other chemicals used in the present study were purchased from Sigma or BDH.

Animal injection

Tranylcypromine (1 mg.kg^{-1}), clorgyline (1 mg.kg^{-1}) or lazabemide (2 mg.kg^{-1}) solutions, dissolved in 0.95% NaCl, were administered daily, to female Sprague-Dawley rats (250–300 g) by intraperitoneal (i.p.) injection. In parallel experiments control animals received 0.95% NaCl. At time-points of 30 min, 7, 14, 21 and 28 days (tranylcypromine study) or 30 min, 4, 8 and 12 days (clorgyline and lazabemide studies) three control and three drug-treated animals, were sacrificed by cervical dislocation. The experiment was repeated on three separate occasions. The time-point of 30 min following the first injection represented day 0 of the treatment schedule.

Sample preparation

Brain, liver and heart were excised and washed in ice-cold 10 mM potassium phosphate buffer, pH 7.5. Brain and liver tissue was homogenised in the same buffer, at a tissue:buffer ratio of 1:10 g.ml^{-1}, using a Dounce homogeniser. Heart tissue was homogenised at the same tissue:buffer ratio using an Ultraturrax (model T-25). Homogenates were then centrifuged at 600 g for 10 min at 4°C. The supernatants were decanted and stored frozen at −20°C in 1 ml aliquots.

Assay of SSAO and MAO activities

Samples were assayed for MAO-A, MAO-B or SSAO activity by a modification of the radiochemical procedure of Tipton (1985). Samples of heart homogenate were assayed for deamination of [^{14}C]-benzylamine (5 μM, 10 Ci.mol^{-1}) using a reaction time of 30 min. Each sample was preincubated at 37°C with 1 mM clorgyline, to inhibit MAO, 1 mM semicarbazide, to inhibit SSAO, or no inhibitor (control), for 30 min prior to assay. MAO-A activity in liver and brain homogenates was measured towards [^{14}C]-5-HT (100 μM, 4.5 Ci.mol^{-1}), using a reaction time of 20 min. The activity of MAO-B was measured towards [^{14}C]-PEA (20 μM, 2 Ci.mol^{-1}), with a reaction time of 10 min. In each case, the reaction was terminated by the addition of citrate and the radiolabelled product was extracted into 4 ml toluene:ethyl acetate (1 : 1,v/v), containing 0.6% 2,5-diphenyloxazole, for liquid scintillation. In all cases, assay conditions used were those determined from preliminary studies to correspond to the linear, initial velocity, period of product formation and a linear dependence of this velocity on the concentration of the tissue sample. Samples from control and experimental animals were assayed in parallel. Protein content of the liver, brain and heart homogenates was assayed in triplicate according to the method of Markwell et al. (1978). For kinetic studies, initial rates were determined by varying the substrate concentration at a constant specific radioactivity and kinetic parameters were determined by non-linear regression.

Western blotting

Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-page) was performed using a 10% resolving gel according to the method of Laemmli (1970). A semi-dry blotting apparatus was used to transfer bands from the gel to nitrocellulose paper (Bio-blot-NC, nitrocellulose blotting membrane). The transfer buffer contained 192 mM glycine, 25 mM Tris-HCl, 1.3 mM SDS and 15% (v/v) methanol, pH 8.3. Transfer was performed at 110 mA for 1 h. After blotting the nitrocellulose was washed with Tween-Tris-buffered saline (TTBS; 20 mM Tris-HCl, 500 mM NaCl, pH 7.5 containing 1 ml.l^{-1} Tween-20). The membrane was blocked with 3% "Marvel" skimmed milk powder in TTBS for 1 h at room temperature. The blot was rinsed thoroughly with TTBS and incubated for 1 h in primary antibody (rabbit anti-bovine SSAO, 1/100 dilution in 3% Marvel/TTBS). Further blot development was carried out using a "Vectastain" avidin/biotin-complex (ABC) kit, according to the manufacturer's instructions. At each time-

point, 6 heart homogenates (3 from control animals and 3 from experimental animals) were diluted to give final protein concentrations of 2, 4 and 6 $mg.ml^{-1}$ and these samples were used for electrophoresis and blotting. Densitometry of Western blots was performed using the GDS8000 Gel Documentation and Analysis System (UVP) which included the GRAB-IT™ and GELWORKS 1D computer programs. The densitometry software determined the intensity of each SSAO band and calculated, in arbitrary units, the intensity of the bands relative to the background intensity.

HPLC of biogenic amines

Reverse-phase, ion-pair HPLC, with electrochemical detection, was used to separate and quantitate dopamine (DA) and its metabolites homovanillic acid (HVA), methoxytyramine, dihydroxyphenyl acetic acid (DOPAC) and 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in brain samples from control and tranylcypromine-treated rats. The levels of PEA were too low to be detected reliably using the HPLC method. The system used, consisted of a C18 μ-Bondapak reverse-phase chromatography column (Waters) connected to a pump (Beckman 110B solvent delivery module) which was operated at a flow-rate of 1 $ml.min^{-1}$. Elution of amines and their metabolites was monitored electrochemically (ESA Coulochem detector 5100). A guard-cell located upstream of the column was set to +0.15 volts, to oxidise interfering components of the mobile phase. The eluted material passed through an analytical cell which comprised two electrodes, one which was set to +0.15 volts to oxidise remaining contaminants in the sample and a second which was set to +0.45 volts, which was optimal for selective oxidation of the components of interest. The mobile phase contained 0.1 M sodium dihydrogen phosphate, 1 mM EDTA (dipotassium salt), 4% methanol and 1 mM octanesulphonic acid, pH 3.81. Striatal tissue was homogenised in 1.5 ml ice-cold 0.4 M perchloric acid (PCA) containing 0.25 mM $Na_2S_2O_5$, 0.1 mM EDTA and 25 $ng.ml^{-1}$ 3,4-dihydroxybenzylamine (DHBA), as internal standard. The sample was centrifuged at 10,000 g for 10 min. The supernatant was filtered through 0.2 μm acrodiscs (Whatman) and then injected directly on to the HPLC column to separate and quantify DA, DOPAC, HVA, methoxytyramine, 5-HT and 5-HIAA. Metabolite concentrations were estimated by reference to standard curves relating concentration of compound to peak-height over a range of 2–10 $ng.ml^{-1}$. Fresh solutions of standards, prepared in 0.4 M (PCA) containing 0.25 mM sodium metabisulphate and 0.1 mM EDTA were chromatographed under the same conditions as the samples.

Results

During long-term (28 day) treatment of rats with the non-selective MAO inhibitor tranylcypromine (1 $mg.kg^{-1}.day^{-1}$), MAO-A and MAO-B activities in rat liver and brain were found to be reduced to between 66–95% of controls, throughout the period (Table 1).

The enzyme activity towards 5 μM benzylamine that remained in rat heart homogenates following preincubation with 1 mM clorgyline was sensitive to inhibition by 1 mM semicarbazide, confirming it to be SSAO. The activity of SSAO in heart homogenates, towards 5 μM benzylamine, was found to be increased to 178 ± 19% of the control activity after 7 days of treatment with tranylcypromine (0.96 ± 0.05 $nmol\,mg^{-1}.h^{-1}$ in control animals, compared to 1.71 ± 0.16 $nmol\,mg^{-1}.h^{-1}$ in the drug-treated animals). At all other timepoints investigated, there were no significant differences in the levels of SSAO activity relative to controls (Fig. 1). In contrast, when rats were treated for 12

Table 1. The inhibition of MAO-A and -B in rat liver and brain after treatment in vivo with MAO inhibitors

a

Duration of treatment (day)	Tranylcypromine treated			
	% inhibition of MAOA		% inhibition of MAO-B	
	Liver (n = 9)	Brain (n = 3)	Liver (n = 9)	Brain (n = 3)
0	83.52 ± 2.31	76.94 ± 2.03	94.81 ± 4.45	74.12 ± 2.33
7	84.82 ± 2.79	91.00 ± 3.78	74.61 ± 4.77	85.00 ± 2.62
14	73.96 ± 15.2	89.00 ± 2.51	66.21 ± 3.32	87.32 ± 4.17
21	86.11 ± 2.13	92.83 ± 1.39	71.02 ± 4.65	83.21 ± 2.03
28	91.12 ± 4.73	91.14 ± 4.73	79.71 ± 4.06	72.12 ± 2.68

b

Duration of treatment (day)	Clorgyline treated		Lazabemide treated	
	% inhibition of MAO-A (n = 3)	% inhibition of MAO-B (n = 3)	% inhibition of MAO-A (n = 3)	% inhibition of MAO-B (n = 3)
0	86.58 ± 7.91	<2	15.30 ± 7.02	90.62 ± 0.49
4	81.62 ± 9.27	<2	<2	90.12 ± 2.25
8	69.93 ± 8.90	7.11 ± 4.0	<2	78.64 ± 2.57
12	93.31 ± 1.63	<2	9.14 ± 5.64	83.83 ± 2.32

The inhibition of MAO-A and MAO-B activities in liver and brain homogenates was monitored a fixed times during the treatment of rats with tranylcypromine at an i.p. dose of 1 mg.kg^{-1}.day^{-1} (**a**). The selective inhibition in rat liver homogenates, of MAO-A activity during treatment with clorgyline (1 mg.kg^{-1}.day^{-1}) and the selective inhibition of MAO-B with lazabemide (2 mg.kg^{-1}.day^{-1}) (**b**). The level of inhibition in the treated groups is expressed as a percentage of control activity. The specific activities for rat brain MAO-A and -B before treatment were 252 ± 14.9 and 76.3 ± 10.3 pmol.min^{-1}.mg protein^{-1} (n = 5), respectively, and those for the rat liver enzymes were 2.98 ± 0.13 and 3.11 ± 0.17 nmol. min^{-1}.mg protein^{-1} (n = 3). Data are expressed as the mean values ± s.e. ratio for the number of animals indicated

days in-vivo with either clorgyline, as a selective inhibitor of MAO-A activity, or with lazabemide, as a selective inhibitor of MAO-B activity, the levels of SSAO activity in heart homogenates were found not to vary significantly from control values at any of the time-points studied (Fig. 2). As shown in Fig. 3, tranylcypromine was a relatively poor inhibitor of SSAO in rat heart homogenates in vitro. The concentration resulting in 50% inhibition (IC_{50}) was determined to be 88.1 ± 0.64 μM. There was no significant inhibition of the SSAO activity in rat heart homogenates by tranylcypromine concentrations in the range of 0.2–1 μM, which gave 65–90% inhibition of the rat liver MAO-A and MAO-B activities in vitro.

The V_{max} value of SSAO towards benzylamine on day 7 of the tranylcypromine treatment was increased from to the control group value of 1.12 ± 0.19 nmol.mg^{-1}.h^{-1} to 2.04 ± 0.35 nmol mg^{-1}.h^{-1} ($P < 0.05$). In contrast, there was no significant change in the K_m value towards this substrate (13.09 ±

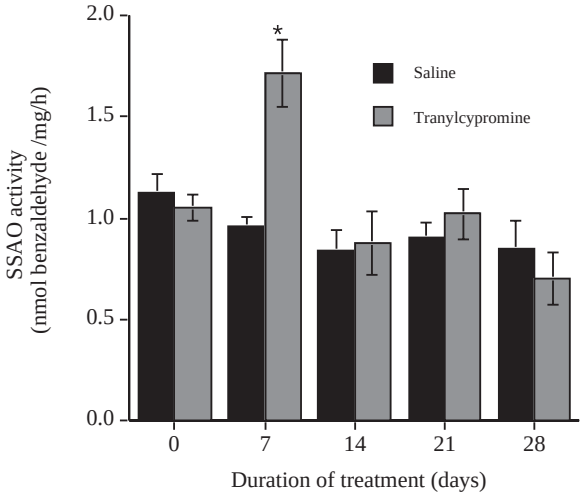

Fig. 1. Activities of SSAO in heart homogenates from rats treated with tranylcypromine compared to controls. Female Sprague-Dawley rats were given daily i.p. injections of 1 mg.kg^{-1} tranylcypromine. In parallel experiments, control animals were injected with 0.95% NaCl. The activities of SSAO towards 5 μM [^{14}C] benzylamine was measured in heart homogenates on the days indicated. Results are expressed as mean values ± s.e.m of 9 animals per group (3 × 3 experiments). *$P < 0.001$ (Student's t-test)

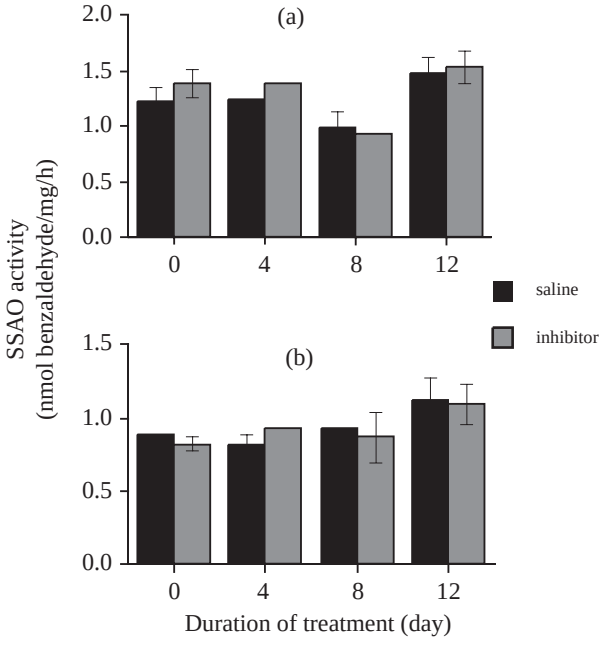

Fig. 2. Activities of SSAO in heart homogenate from rats treated in vivo with clorgyline or lazabemide. Female Sprague-Dawley rats were given daily i.p. injections of **a** clorgyline (1 mg.kg^{-1}) or **b** lazabemide (2 mg.kg^{-1}). In parallel experiments, control animals were injected with 0.95% NaCl. The activity of SSAO towards 5 μM [^{14}C] benzylamine was measured in heart homogenates as described in the text. Results are expressed as mean values ± s.e.m of triplicate determinations carried out on 3 animals per group

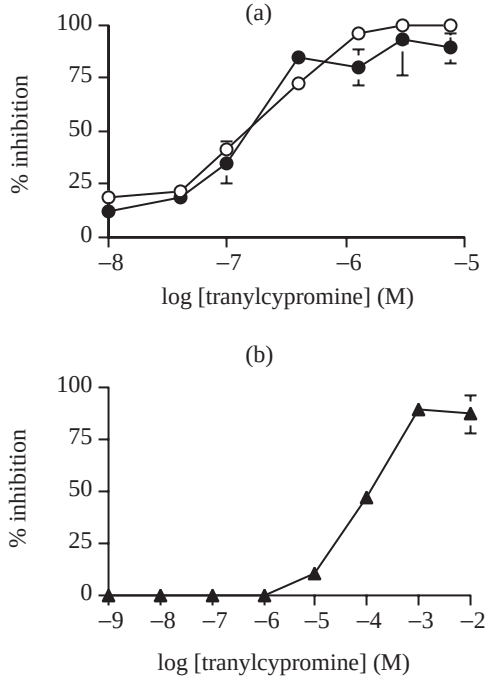

Fig. 3. The effect of tranylcypromine concentration on the activities of **a** MAO-A (○) and -B (●) in rat liver homogenate and **b** SSAO rat heart homogenate. Homogenates were preincubated for 30 min with different concentrations of tranylcypromine, before activity was determined. Each point represents the mean value ± range of duplicate determinations from a representative experiment

2.23 μM and 11.69 ± 2.63 μM for control and 7-day tranylcypromine groups, respectively; Fig. 4). At other times during the treatment schedule, there was no significant difference in either kinetic parameter between control and tranylcypromine-treated groups (Fig. 4). The increased V_{max} value of the 7-day tranylcypromine-treated samples was reflected by a visible increase in SSAO immunoreactivity (Fig. 5). Densitometric analysis showed the mean increase of 185% ± 49% in the SSAO band intensity, compared to controls; a value similar to the increase in SSAO activity at that time.

Although there were no significant differences in the K_m values towards benzylamine between control and tranylcypromine-treated samples, the K_m value of SSAO in heart homogenates from both control and treated samples decreased over the course of this study, as shown in Fig. 4. The initial K_m values (30 min after the first injection) were 16.6 ± 3.5 μM in the control group and 20.7 ± 4.7 μM in the tranylcypromine-treated group. By day 7 of treatment the K_m values in both control and treated groups had fallen to 13.1 ± 2.2 μM and 11.7 ± 2.6 μM, respectively. The K_m values of both control and tranylcypromine-treated samples were <6 μM on all subsequent days studied; significantly lower than those at either day 0 and day 7 ($P < 0.05$). These changes appear to represent elevated K_m values over the first 7 days of treatment, since the values for both the control and drug-treated animals on days

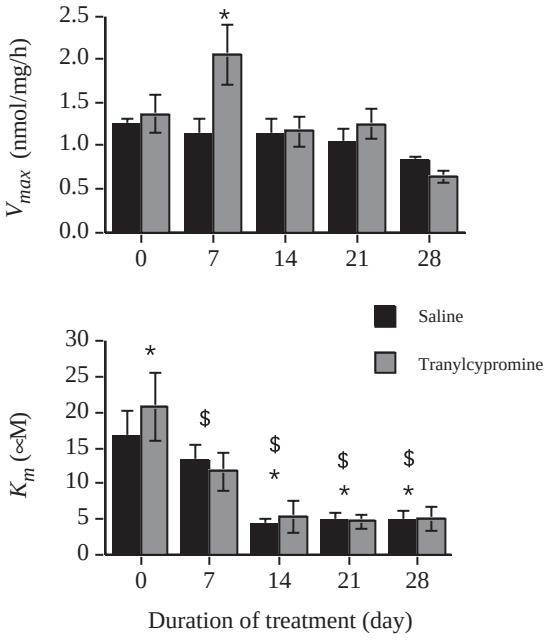

Fig. 4. Maximum velocity and Michaelis parameters for benzylamine oxidation by rat heart homogenates from control and tranylcypromine-treated rats. Results are expressed as the mean value ± s.e.m of determinations from 4 animals selected from 2 separate experiments. *and $ P-value <0.05 (by Student's t-test). Note *compares days 14, 21 and 28 to day 0, $ compares days 14, 21 and 28 to day 7

14, 21 and 28 of the treatment schedule were similar to those from animals which had received no injection (4.70 ± 0.56μM, n = 3). The significantly elevated K_m values for SSAO found on day 0 (30min after injection) and day 7 of the tranylcypromine study, may have resulted from the presence of an endogenous inhibitor. However, extensive dialysis of heart homogenates from

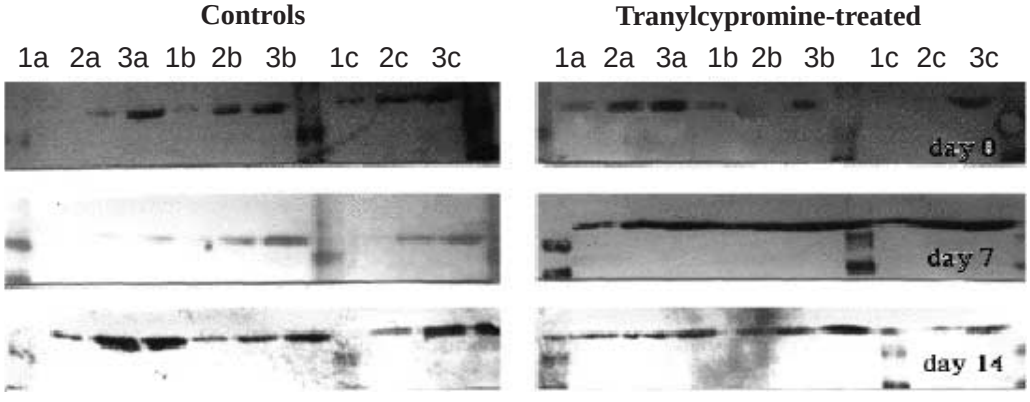

Fig. 5. Immunoblots of rat heart homogenates from control and tranylcypromine-treated animals from day 0, 7 and 14 of study. Each homogenate was blotted at 2mg.ml^{-1} (1), 4mg.ml^{-1} (2) and 6mg.ml^{-1} (3) protein. In each case, a, b and c refer to homogenates from three different animals

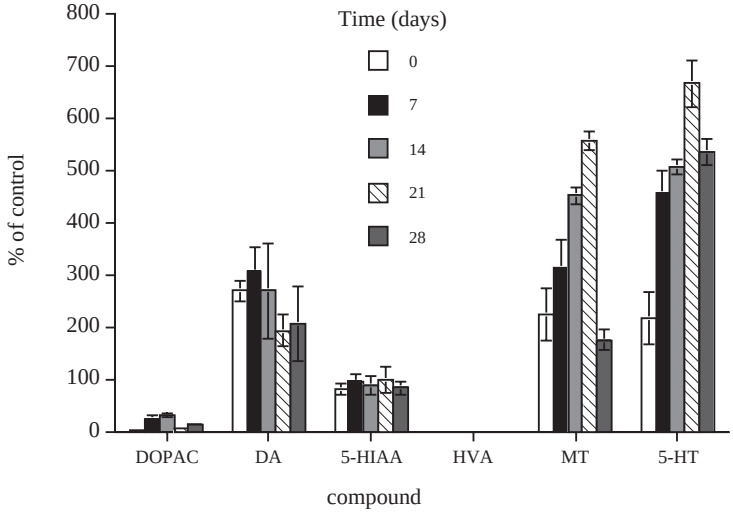

Fig. 6. Levels of amines and amine metabolites in striata from rats treated in vivo with tranylcypromine. The levels of dopamine and its metabolites (DOPAC, HVA and methoxytyramine) and 5-HT and its metabolite 5-HIAA, were measured in the striatum of rat brains from the tranylcypromine study using HPLC with electrochemical detection. Amine and amine metabolite levels at each time-point are expressed as a percentage of the control level ± s.e.m. (n = 3)

day 0 or day 28 of this study did not significantly alter the elevated K_m values for benzylamine oxidation in heart homogenates at either time-point (data not shown).

Figure 6 shows that the levels of dopamine in the striata of tranylcypromine-treated animals were increased at all of the time-points studied, to between 195–309% of control levels, whereas the levels of its oxidatively deaminated metabolite, DOPAC, were decreased to between 3–34% of controls. HVA was not detectable in samples from tranylcypromine-treated rats. The levels of 3-methoxytyramine, the immediate O-methylated derivative of dopamine were found to increase gradually, when compared to controls, up to the third week of drug treatment. After this time, the levels of this amine were found to decrease to values below those found in week one. Thus it appears that the metabolism of dopamine was severely affected, during long-term MAO-A and MAO-B inhibition. The levels of 5-HT were also found to increase during tranylcypromine treatment, reaching a maximum of 666% of the control value on day 21 of treatment. However the levels of 5-HIAA, the oxidatively deaminated metabolite of 5-HT, were not significantly altered in the treated samples compared to controls, despite the disruption to normal 5-HT metabolism caused by MAO inhibition.

Discussion

The transient increase in rat heart SSAO activity resulting from inhibition of MAO-A and MAO-B by treatment with tranylcypromine in vivo reflected a change in the V_{max} value towards benzylamine with no significant change in

the K_m value. The increase of SSAO activity found in these "ex-vivo" studies was paralleled by a change in the amount of SSAO immunoreactive material found in Western-blot analysis. The half-life value for new enzyme synthesis, estimated following the treatment of rat aortic smooth muscle cells with the irreversible SSAO inhibitor MDL72145, was found to be approximately 6 days (Fitzgerald et al., 1998), which is longer than the "ex-vivo" half-lives of approximately 60 h and 70 h reported for rat aorta and rat lung SSAO, respectively (Palfreyman et al., 1994). An increase in the activity of human plasma SSAO in vivo in response to the infusion of dopamine has also been reported, but this was more rapid, being significant after 1 h and reaching a maximum after 2 h (Zis et al., 1980).

The increase in the activity of SSAO found in the present study may result from an increase in the expression of this protein or from an alteration in the enzyme synthesis/degradation ratio. Alternatively the enzyme may be released from other sites such as blood vessels. It has been reported that an endogenous inhibitor of SSAO is present in human plasma (Buffoni et al., 1983) but the results of the Western-blot analysis suggest that the increased activity reflects an increase in the amount of detectable enzyme. The failure of dialysis to affect the SSAO activity would suggest that any endogenous modulator, if present, would be a relatively large molecule and the possibility of such an inhibitor also masking sites recognised by the polyclonal antibodies used cannot be excluded.

The transient increase in rat heart SSAO activity was found only when a non-selective (MAO-A and MAO-B) inhibitor was used, whereas the selective inhibitors, clorgyline and lazabemide were without effect. Some substrates such as 5-HT, PEA and tyramine have been found to be oxidised by both MAO isoforms, although with different K_m and V_{max} values (Suzuki et al., 1981; Kinemuchi et al., 1980; Tipton et al., 1982). Thus, MAO-A can oxidise many preferential MAO-B substrates when these are present at sufficiently high concentrations and, similarly, MAO-B can metabolise substrates preferentially oxidised by MAO-A when their concentrations are high. Therefore, the amine balance of an organism in which one or other of the MAO isoenzymes is inactive might not be as severely disrupted as it would be if both isoenzymes were inhibited. Indeed, it has been reported that the alterations in the levels of the trace amines were less severe in individuals with a genetically determined deficiency of one or other form of MAO than in individuals with combined deficiency of MAO-A and MAO-B (Lenders et al., 1996). Thus changes in SSAO that may, in part, compensate for a loss of MAO activity may be effected only when the activities of both MAO-A and MAO-B are significantly impaired.

The SSAO activity in rat heart homogenates was inhibited by tranylcypromine in vitro with an IC_{50} value of 88 μM. However, the doses of tranylcypromine administered in these experiments would not be expected to produced such a high in vivo concentration of tranylcypromine. The possibility that a metabolite of tranylcypromine might activate SSAO cannot be excluded, although the results of dialysis experiments would suggest any such effects to be not readily reversible. Studies on the effects of tranylcypromine

metabolites on SSAO might be of value in differentiating between the possibility that the transient activation of SSAO was in response to the drug itself or to the elevated concentration of MAO-A and -B substrates following the inhibition of these enzymes.

SSAO activity is absent from brain tissue, except for the cardiovascular enzyme present in microvessels. This may explain why the changes in SSAO activity, resulting from tranylcypromine treatment, did not appear to affect the alterations of DA, 5-HT and their metabolites in the striatum resulting from MAO inhibition. The oxidative deamination of dopamine was severely disrupted, reflecting the inhibition of MAO-A and -B activities and no significant contribution from blood-vessel SSAO in striatal dopamine oxidation was evident from amine and metabolite analysis. The increase in methoxytyramine found in tranylcypromine-treated animals, compared to controls, indicated a shift to the O-methylation pathway for dopamine metabolism, which has also been reported to occur in atypical Norrie diseased patients (Murphy et al., 1990; Lenders et al., 1996). The finding that 5-HT levels increased in response to MAO inhibition, whereas its oxidatively deaminated metabolite 5-HIAA was not significantly affected, is consistent with the effects reported in Norrie disease (Murphy et al., 1990) and in depressed individuals treated with phenelzine (McKenna et al., 1993). Except in dental pulp (Norqvist and Oreland, 1989; Norqvist et al., 1982), SSAO is not active towards 5-HT and, furthermore, the levels of 5-HIAA found in the treated animals, were independent of the transient induction of SSAO activity in the rat heart.

The sequence of SSAO, at the DNA level, has been reported to be identical with that of vascular-adhesion protein1 (VAP1), which has a key role in mediating inflammation (Smith et al., 1998). It is interesting that an increase in the K_m value of rat heart SSAO towards benzylamine occurred on days 0 (30 min) and 7 of treatment, in both control and tranylcypromine-treated animals. The only common feature in these two groups of animals was the i.p injection (saline in controls, tranylcypromine in treated groups). Although it has been shown that i.p. injection per se does not elicit any significant inflammatory response as judged by analysis of leukocyte-endothelial cell interaction (Tohka et al., 2001), it is possible that the increased K_m values represent a response to stress due to daily injection in the earlier days of this study. Taken together, the changes in the activity and behaviour of heart SSAO reported here suggest that, not only is this activity induced in response to MAO inhibition/excess amines, but the enzyme activity may have further important functions in the vasculature, particularly in the processes of cellular stress and inflammation. The finding that the VAP1 protein has SSAO activity which may be involved in regulating the levels of bioactive amines at sites of inflammation, has linked two important research fields and will provide useful insight into the previously elusive roles of SSAO.

Acknowledgements

We are grateful to the European Commission and the Health Research Board of Ireland for grants in support of this work and to Dr. G. Richards (Hoffman la Roche Laborato-

ries, Basel, Switzerland) and Drs. M. Unzeta and J.-M. Lizcano (Autonomous University of Barcelona, Spain) for their kind gifts of lazabemide and anti-SSAO antibodies.

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