J. Pharm. Pharmacol. 1995, 47:837–845
Received December 13, 1994
Accepted July 7, 1995

Further Studies on the Ex-vivo Effects of Procarbazine and Monomethylhydrazine on Rat Semicarbazide-sensitive Amine Oxidase and Monoamine Oxidase Activities

ANDREW HOLT* AND BRIAN A. CALLINGHAM

Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, UK

Abstract

Following administration of the anticancer agent, procarbazine, or one of its metabolites, monomethylhydrazine, to rats, activities of monoamine oxidases A and B (MAO A and MAO B) and of semicarbazide-sensitive amine oxidase (SSAO) were measured ex-vivo.

Both compounds were found to be potent inhibitors of SSAO in tissue homogenates, exhibiting ID50 values in most tissues of approximately 8 mg kg^{-1} (procarbazine) and 0·08 mg kg^{-1} (monomethylhydrazine). Concurrent dose-dependent inhibition of MAO activities did not occur. However, in liver, potentiation of MAO B activity, to 140% of that in controls, was apparent following monomethylhydrazine and this effect was independent of the drug dose. Both compounds produced a dose-dependent potentiation of MAO A in brown adipose tissue, the elevation being more pronounced following monomethylhydrazine, with activity rising to 350% of that in control homogenates. In a parallel in-vitro study, monomethylhydrazine was without effect on MAO A in brown adipose tissue homogenates. By perfusing the SSAO substrate, benzylamine, through the isolated mesenteric arterial bed of the rat, it was found that pretreatment of animals with procarbazine or monomethylhydrazine reduced metabolism of this amine by a similar degree as had been determined ex-vivo in blood vessel homogenates.

The results presented suggest that these compounds would be suitable for use as selective inhibitors in pharmacological examinations of SSAO function in isolated tissues and organs.

Most semicarbazide-sensitive amine oxidase enzymes [amine: oxygen oxidoreductase (deaminating) (copper containing); EC 1.4.3.6; SSAO] catalyse the oxidative deamination of primary monoamines and have, like monoamine oxidase (MAO), a wide distribution throughout the animal and plant kingdoms. Although these two enzyme groups can be separated on the basis of primary structures which are entirely distinct (Bach et al 1988; Klinman & Mu 1994 and references therein), a number of substrates and inhibitors common to both can be demonstrated in-vitro. However, while the contribution of MAO towards, for example, the metabolism of ingested amines or the regulation of neuronal amine transmitter content have been well documented, an endogenous SSAO substrate has, thus far, proved elusive and consequently, most SSAO enzymes have yet to be ascribed a physiological function. Our failure in this regard has been compounded by a lack of potent, selective SSAO inhibitors. The term, SSAO, encompasses both copper-containing amine oxidases, including plasma amine oxidases and diamine oxidase, and those tissue-bound enzymes where the presence of copper has not been confirmed (see Callingham et al 1991). An absence of copper may indicate differences in cofactor requirement between these SSAO subgroups (Matsuzaki et al 1994). Nevertheless, all SSAO subtypes appear to contain a carbonyl cofactor of some description (Klinman & Mu 1994) and, as a result, these enzymes are particularly sensitive to inhibition by hydrazine-based compounds (Lyles 1984).

In species where comprehensive studies of SSAO distribution have been performed, highest enzyme activity was found in vascular and other tissues composed predominantly of smooth muscle (Coquil et al 1973; Lewinsohn 1981, 1984) and in brown (Barrand et al 1984) and white (Raimondi et al 1991) adipocytes. In vascular and fatty tissues at least, the predominant subcellular association of SSAO would appear to be with the plasmalemma (Wibo et al 1980; Barrand & Callingham 1982, 1984; Nakos & Gossrau 1994); this is in contrast to the mitochondrial location of MAO. In rat blood vessels, it is likely that most, if not all, the SSAO active sites face outwards (Holt & Callingham 1993, 1994a).

The localization of high SSAO activity within the plasmalemmae of vascular smooth muscle cells makes this enzyme ideally situated to scavenge circulating amines and compounds such as methylamine (Precious et al 1988; Lyles et al 1990), aminoacetone (Lyles & Chalmers 1992; Lyles 1994) and histamine (Buffoni et al 1994) have been discussed as possible endogenous substrates. However, SSAO in adipocytes seems less well suited to such a role and enzyme function might therefore be linked to enzyme location. Thus, there may exist more than one physiological substrate, although benzylamine, which is metabolized more rapidly by most SSAO enzymes than are other amines, has not yet been detected in mammalian tissues.

In attempting to elucidate the physiological role of SSAO, it may help to examine the effects of specific inhibitors of these enzymes on the responses of isolated tissues and

* Present address and correspondence: A. Holt, Neurochemical Research Unit, Department of Psychiatry, 1E7·44 Mackenzie Health Sciences Centre, University of Alberta, Edmonton, AB, T6G 2B7, Canada.

organs to pharmacological and electrical stimuli and to perfused substrates (for example, see Elliott et al 1989a, b). Inhibitors can be added to organ baths or to fluids perfusing whole organs. However, to obtain a more accurate indication of the effects upon SSAO activities of drug administration to the whole animal, it is necessary to conduct experiments ex-vivo.

It has been demonstrated previously that the carcinostatic agent, procarbazine and two of its metabolites, azoprocarbazine and monomethylhydrazine exhibit tremendous selectivity for SSAO in-vitro (Holt et al 1992 a, b). It seems unlikely that either of the two other major metabolites of this drug which occur in-vivo, *p*-formyl-*N*-isopropylbenzamide or *N*-isopropyl terephthalamic acid, would have a high affinity for the MAO active site. Therefore, unless one or more of the reactive, transient intermediates generated during metabolism of procarbazine is able to cause substantial inhibition of MAO, then it is probable that the selectivity of procarbazine and its metabolites will be retained ex-vivo. To test this hypothesis, the effects have been examined of procarbazine and monomethylhydrazine on amine oxidase activities in homogenates of various tissues following intraperitoneal administration of these compounds to rats. Inhibition of benzylamine metabolism was also quantified following perfusion of benzylamine through isolated superior mesenteric arterial beds dissected from drug-treated rats (Elliott et al 1989a). The term ex-vivo, has been used to classify procedures where drugs were administered prior to death and removal of tissues for experimentation. Preliminary findings from some of these studies have already been published (Holt & Callingham 1994b).

Materials and Methods

Materials

Substrates for the radiochemical amine oxidase assays were [7-^{14}C]benzylamine hydrochloride, from Dupont UK Ltd (Stevenage, Herts, UK) and 5-hydroxy[G-^{3}H]tryptamine creatinine sulphate, from Amersham International plc (Amersham, Bucks, UK). These were diluted with appropriate amounts of unlabelled amines to obtain stock solutions of suitable concentration and specific activity for use in enzyme assays. Unlabelled benzylamine was obtained as the free base from Sigma Chemical Co. (Poole, Dorset, UK) and crystallized as the hydrochloride in the laboratory. Unlabelled 5-hydroxytryptamine creatinine sulphate was obtained from Sigma. Opti-fluor O (liquid scintillation fluid) was obtained from Canberra Packard (Pangbourne, Berks, UK). Procarbazine, a gift from Hoffmann-La Roche (Basel, Switzerland) and monomethylhydrazine, from Aldrich Chemical Co. (Gillingham, Dorset, UK), were obtained as the free bases and dissolved with an equivalent amount of HCl in aqueous solution. Sagatal was obtained from RMB Animal Health Ltd (Dagenham, Essex, UK) and heparin BP from Paines & Byrne Ltd (Greenford, Middlesex, UK). (−)-Noradrenaline, obtained as the bitartrate salt, was purchased from Sigma. All other reagents were of analytical grade, where possible.

Male Wistar rats were obtained from the departmental breeding colony.

Modified Krebs–Henseleit solution, prepared in distilled water, had the following composition (mM): NaCl 118, KCl 4·57, $CaCl_2$ 1·27, KH_2PO_4 1·19, $MgSO_4$ 1·19, $NaHCO_3$ 25 and D-glucose 5·55. When this solution was used as a solvent in the preparation of noradrenaline, L-ascorbic acid (116 μM) and EDTA (41 μM) were also included.

Preparation of tissue homogenates

Male Wistar rats, 60–185 g for tissue homogenate experiments and 280–365 g for perfusion studies, were maintained on a 12-h light–dark cycle, during which they were allowed free access to drinking water and a standard laboratory rat diet, until required for experimentation.

When tissues obtained were to be homogenized, animals received intraperitoneal injections of 0·9% (w/v) saline (controls), procarbazine (0·1–100 mg kg^{-1} in saline) or monomethylhydrazine (1 μg kg^{-1}–1 mg kg^{-1} in saline), in injection volumes of 10 or 20 mL kg^{-1} bodyweight. Animals were killed by stunning and decapitation, 2 h after injection. The brain and heart were removed from each animal, along with tissue dissected from caudal lobes of livers and lungs. Pads of interscapular brown adipose tissue (BAT) and the entire thoracic portion of the aorta posterior to the aortic arch were also dissected out. All tissues were immediately stored at −20°C until required and amine oxidase activities in tissue homogenates were measured within 10 days of tissue removal. Homogenates were prepared in 0·5 mM potassium phosphate buffer, pH 7.8, with a Polytron Mark 5 mechanical homogenizer (Kinematica). The final tissue (g) to buffer (mL) ratios were as follows: 1 : 80 (aorta), 1 : 40 (BAT), 1 : 10 (brain), 1 : 20 (heart), 1 : 20 (liver) and 1 : 20 (lung). At these ratios, rates of substrate metabolism were linear for the period of incubation under the conditions used. All homogenates were prepared on the day of assay.

Preparation of mesenteries for perfusion

When the metabolism of perfused benzylamine by SSAO in rat mesenteric arteries was to be examined, procarbazine and monomethylhydrazine were administered to rats as an intraperitoneal injection of 10 mL kg^{-1} bodyweight. Animals received 100 mg kg^{-1} procarbazine or 1 mg kg^{-1} monomethylhydrazine, while control animals received 1 mL kg^{-1} saline. These drug doses had been shown to cause almost complete inhibition of SSAO in rat aortae ex-vivo after 2 h (see Results). Two preparations were examined at one time.

Two hours after injection of drugs, animals were anaesthetized with sodium pentobarbitone (Sagatal, 90 mg kg^{-1}) and heparinized (500 units) in a single intraperitoneal injection. The mesenteric arterial bed was then isolated and perfused using a technique based on that of McGregor (1965), as described by Elliott et al (1989a). Briefly, the superior (cranial) mesenteric artery was cleaned of adhering tissue and ligated with a cotton thread, close to the abdominal aorta. The artery was cannulated with a polyethylene catheter (1·02 mm o.d., Portex Ltd, Hythe, Kent) and, after the catheter had been tied in place with a second ligature, perfusion with modified Krebs–Henseleit was started immediately. The Krebs solution was maintained at 37°C and was gassed with 95% O_2–5% CO_2; the pH of this solution was 7·4. The mesentery was then parted from the intestine and attachments to the liver, spleen and pancreas were severed.

The isolated mesentery was placed in a methacrylate dish on a perspex block warmed by water circulating at 37°C and the preparation covered with paraffin film (Nescofilm, Nippon Shoji Kaishi Ltd, Osaka, Japan) to prevent evaporation. A constant flow rate of 2 mL min^{-1} was maintained by a Harvard Model 1203A peristaltic pump. Pressure within each system could be monitored with a Washington PT400 pressure transducer, located close to the mesentery, and transducers were connected via an amplifier to a Kipp & Zonen BD41 two-channel X/T pen recorder.

Measurement of amine oxidase activities in homogenates

Amine oxidase assays were based on the method described by Lyles & Callingham (1982).

Assays for SSAO, MAO A and MAO B were performed separately, in triplicate, in ice-cooled soda glass tubes, containing 50 μL of tissue homogenate and 50 μL of the appropriate radiolabelled substrate. Substrates used were, for SSAO, [^{14}C]benzylamine (10 μM, sp. act. 10 μCi μmol^{-1}), for MAO A, [^{3}H]5-hydroxytryptamine (250 μM, sp. act. 2 μCi μmol^{-1}) and for MAO B, [^{14}C]benzylamine (250 μM, sp. act. 1 μCi μmol^{-1}). Tubes were flushed with oxygen, stoppered and samples incubated at 37°C for 10 min. Enzyme activities were terminated by plunging tubes into ice and adding HCl (3 M, 10 μL) to each. Addition of acid also served to ensure that unreacted amine substrate was protonated while labelled metabolites remained uncharged. Blank tubes had HCl added before incubation with substrate. Deaminated metabolites were extracted into 700 μL ethyl acetate/toluene (1 : 1, v/v), saturated with water (extraction efficiency 99% for benzylamine and 87% for 5-hydroxytryptamine) and 400 μL of the organic phase was counted for radioactive metabolites in 4 mL of Opti-fluor O liquid scintillation fluid in a scintillation spectrometer (Packard model 2500 TR), with quench correction by automatic external standardization.

Metabolism of perfused benzylamine

After consistent pressor responses had been established to the administration via injection ports of 25 nmol noradrenaline, both tissues were perfused with [^{14}C]benzylamine (25 μM, 2 μCi μmol^{-1} in Krebs solution) for 5 min followed by Krebs alone. Vascular resistance (perfusion pressure) did not change as a result of perfusion with benzylamine. Eluate was collected from each preparation into soda glass disposable tubes, at 15-s intervals for 15 min. The total radioactivity and radioactivity due to metabolites of benzylamine were then measured in each fraction. Total radioactivity was measured by sampling 20 μL into 2 mL ethoxyethanol and counting for radioactivity in 10 mL Opti-fluor O. The remainder of each fraction was acidified by adding HCl (3 M, 50 μL), deaminated metabolites were extracted into 2·5 mL ethyl acetate/toluene and 2 mL of the organic phase was counted for radioactive metabolites in 10 mL Opti-fluor O, as described above. The summation of metabolite levels measured in fractions obtained when benzylamine was perfused through the apparatus in the absence of a mesentery constituted a blank reading for these experiments.

Protein assays

Protein contents of homogenates were determined by the method of Lowry et al (1951), with bovine serum albumin as standard.

Statistical analysis

Values are expressed as the mean ± standard error of the mean (s.e. mean) or as the mean ± standard error of the ratio (s.e. ratio), where appropriate. The number of tissues examined in each group is represented by n. Wilcoxon's two-sample rank test was used to compare amine oxidase activities in tissue homogenates from drug-treated animals with those in homogenates obtained from control tissues. Perfusion experiments were performed in duplicate and mean results, along with the range, are therefore quoted.

Results

Effects of procarbazine and monomethylhydrazine on enzyme activities in homogenates

Following intraperitoneal administration of saline, procarbazine or monomethylhydrazine to rats, various tissues and organs were removed and MAO A, along with either MAO B or SSAO activities were measured in tissue homogenates. Figs 1 and 2 show the ex-vivo effects of procarbazine and monomethylhydrazine pretreatment, respectively, on amine oxidase activities, compared with controls. Control enzyme activities (nmol h^{-1} (mg protein)$^{-1}$, mean ± s.e.mean; n = 5) were as follows: for MAO A, 3·07 ± 0·18 (aorta), 17·93 ± 0·79 (brain), 1·56 ± 0·10 (BAT), 19·69 ± 3·62 (heart), 3·05 ± 0·05 (liver), 8·30 ± 0·75 (lung); for MAO B, 19·82 ± 0·42 (brain), 5·38 ± 0·12 (liver); for SSAO, 1·51 ± 0·05 (aorta), 1·74 ± 0·09 (BAT), 1·73 ± 0·17 (heart), 1·21 ± 0·09 (lung).

Treatment with either procarbazine or monomethylhydrazine resulted in a dose-dependent inhibition of SSAO activities in all tissues examined. Estimated ID50 values obtained from these plots by nonlinear regression (GraphPad Prism, Version 1·03; GraphPad Software, San Diego, CA) for inhibition of SSAO by procarbazine and monomethylhydrazine, respectively, were (mg kg^{-1}): 9·5 and 0·10 (aorta), 5·4 and 0·11 (BAT), 15·8 and 0·16 (heart) and 23·3 and 0·16 (lung). However, low doses of both drugs produced a small, but significant potentiation of benzylamine metabolism in lung homogenates (Figs 1f, 2f). Little inhibition of either form of MAO occurred in most tissues, although MAO A in aortic homogenates displayed some sensitivity to procarbazine (Fig. 1a).

Significantly, treatment with monomethylhydrazine failed to inhibit either form of MAO in the brain and modest inhibition of both brain enzymes was only evident in rats which had received the highest dose (100 mg kg^{-1}) of procarbazine (Fig. 1b).

In rats treated with monomethylhydrazine, significant potentiation of hepatic MAO B occurred in a manner independent of the dose used (Fig. 2e), but potentiation of hepatic MAO B by procarbazine did not reach statistical significance. In contrast, both procarbazine and monomethylhydrazine caused significant, dose-dependent potentiation of MAO A activity in homogenates of BAT (Figs 1c, 2c).

To determine whether the ex-vivo potentiation of MAO A in BAT which occurred was due to a direct drug action on

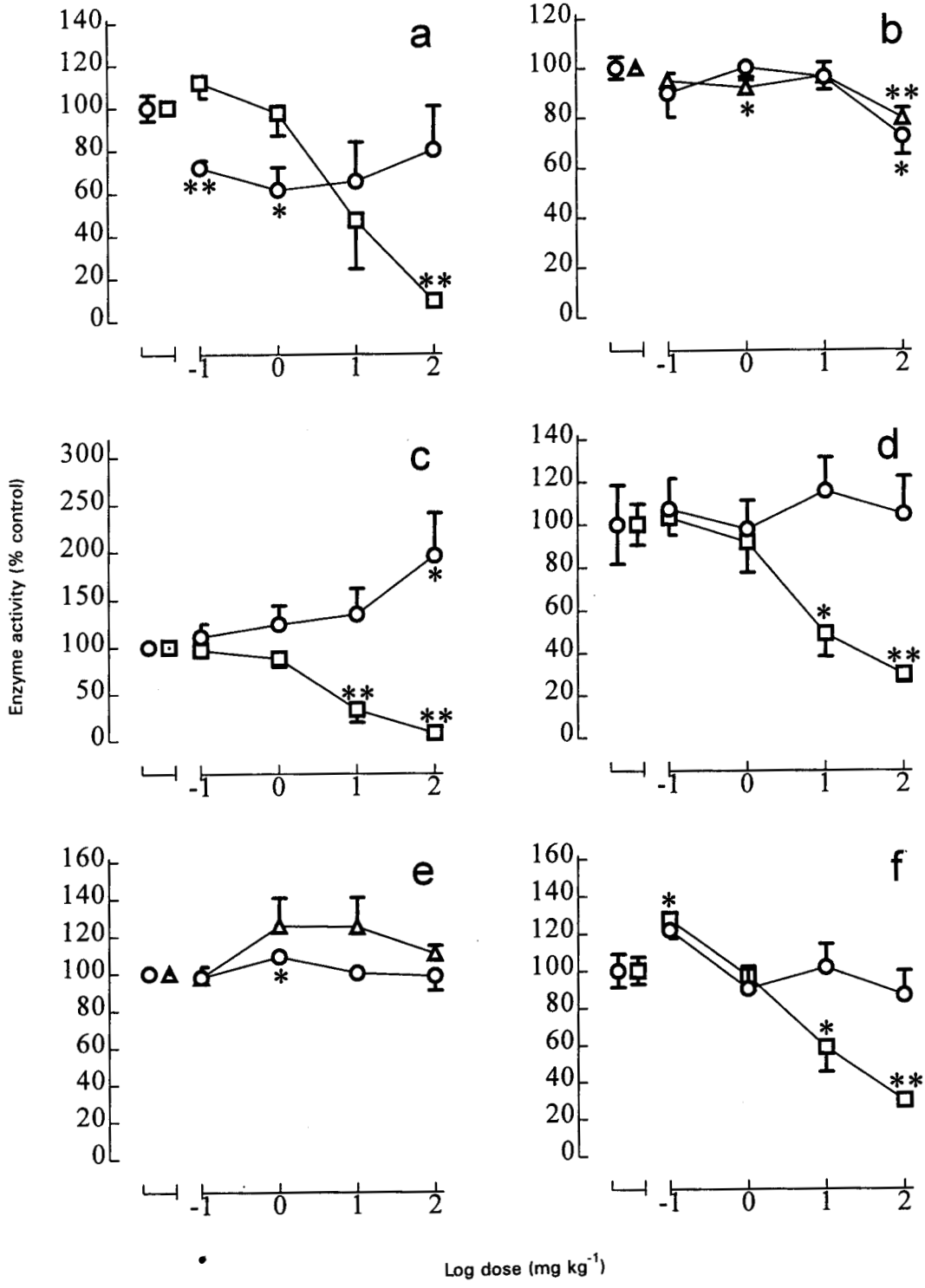

FIG. 1. The ex-vivo effects of procarbazine on SSAO (□), MAO A (○) and MAO B (△) activities in homogenates of rat aorta (a), brain (b), brown adipose tissue (c), heart (d), liver (e) and lung (f). $*P < 0.05$, $**P < 0.01$ compared with control activities (n = 5).

the enzyme, samples of BAT homogenate were preincubated with monomethylhydrazine (0·1 μM) for 2 h and remaining MAO A activities compared with those in control samples, preincubated with water. In BAT homogenates preincubated with monomethylhydrazine, MAO A activity was measured as 97·8 ± 1·9% (n = 3) of that in control samples.

Inhibition of metabolism of perfused benzylamine

Fig. 3 shows the perfusion profiles of that radioactivity in fractions which was due only to metabolites of benzylamine. Also shown is the profile obtained when benzylamine was perfused in the absence of a mesentery. It was determined that, in these experiments, 3·73 nmol of the apparent total

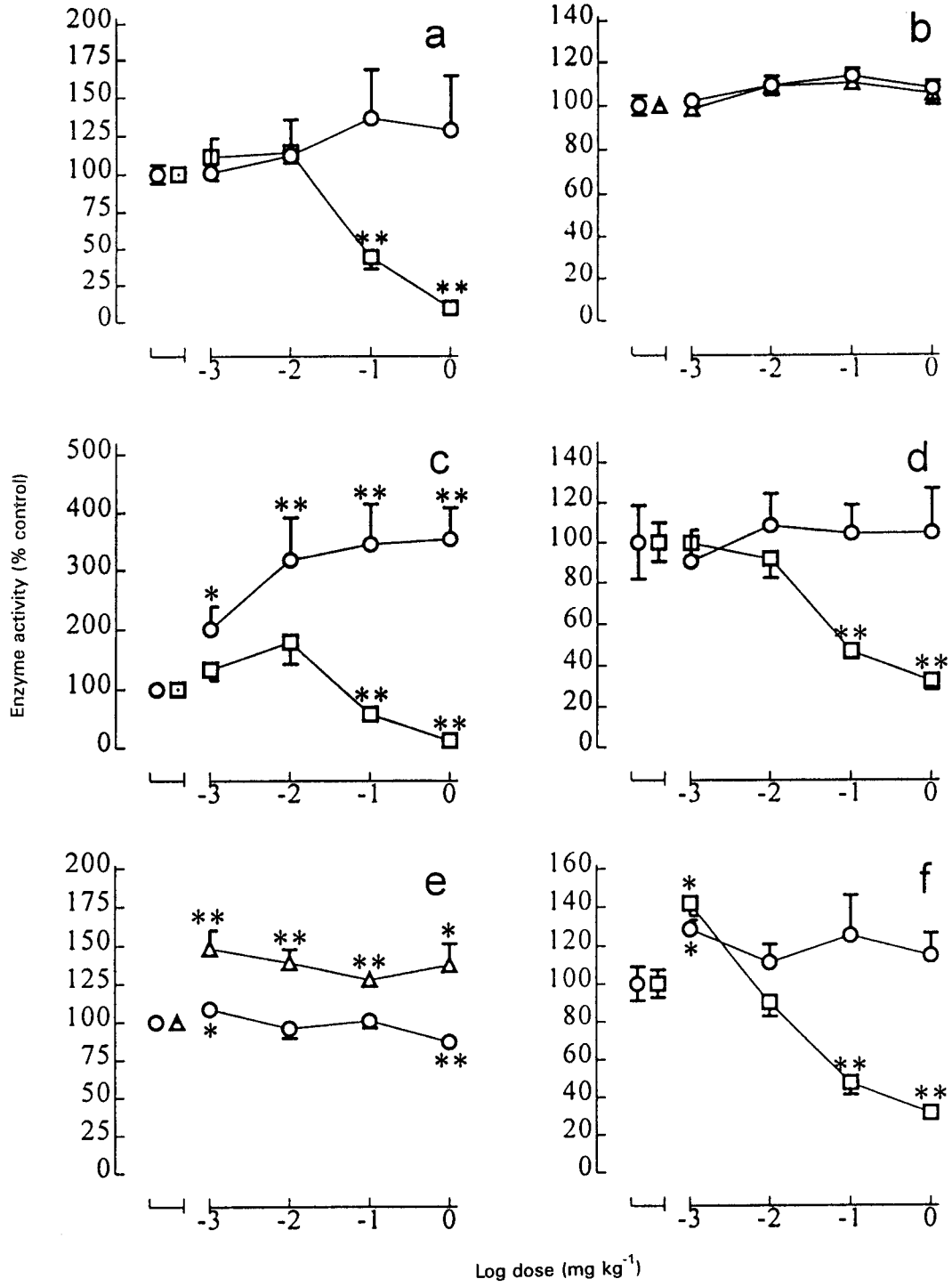

FIG. 2. The ex-vivo effects of monomethylhydrazine on SSAO (□), MAO A (○) and MAO B (△) activities in homogenates of rat aorta (a), brain (b), brown adipose tissue (c), heart (d), liver (e) and lung (f). $*P < 0{\cdot}05$, $**P < 0{\cdot}01$ compared with control activities (n = 5).

metabolite production could be accounted for by the presence of radiolabelled contaminants or degradation products. Quantities of radioactivity recovered and metabolites extracted are shown in Table 1, along with values corrected to take account of blank readings. Almost all of the perfused amine was recovered, of which, in control preparations, approximately 15% was metabolized by SSAO.

Discussion

Following drug administration, pharmacokinetics and drug metabolism will have a profound effect on whether the potency and selectivity of SSAO inhibitors evident in in-vitro studies will be retained in-vivo. Presently, we are faced with a dearth of compounds which do retain their in-vitro selectivity and potency. Phenelzine and benserazide are both

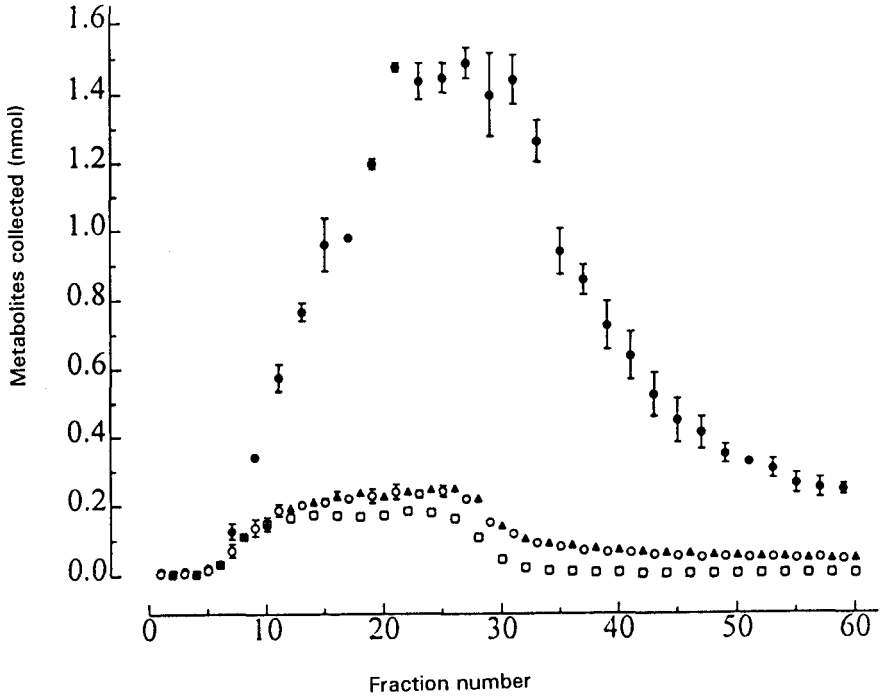

FIG. 3. Effects ex-vivo of procarbazine and monomethylhydrazine on [^{14}C]benzylamine metabolite profiles in the perfusate collected from rat isolated mesenteric arterial beds. ● Control, ○ 100 mg kg^{-1} procarbazine, ▲ 1 mg kg^{-1} monomethylhydrazine, □ blank perfusion of benzylamine in the absence of a mesentery.

selective for SSAO over MAO in in-vitro studies (Andree & Clarke 1982). However, ex-vivo, phenelzine causes substantial MAO inhibition, and while benserazide retains its selectivity for SSAO ex-vivo, its effects are readily reversible by dialysis. Chronic administration of high doses of benserazide is necessary to attain substantial SSAO inhibition in rat BAT (Lyles & Callingham 1982), increasing the likelihood of interactions with other carbonyl-containing enzymes.

Following the intraperitoneal administration of a range of doses of procarbazine or monomethylhydrazine to rats, dose-dependent inhibition of SSAO activities was measured in homogenates of heart, aorta, lung and BAT. No concomitant dose-dependent inhibition of MAO A could be detected in the same tissues and neither MAO A nor MAO B were markedly inhibited in homogenates of liver and brain from the same animals. Only at the highest dose of procarbazine (100 mg kg^{-1}) was brain MAO activity reduced compared with control animals and even then, both forms were inhibited only by around 20%. Low doses of procarbazine (0·1 and 1 mg kg^{-1}) may also have caused a slight, but significant, anomalous reduction in rat aortic MAO A activity. SSAO in heart and lung appeared to be resistant to complete inhibition by either compound, whereas SSAO activity in aorta was abolished almost completely at the highest doses of both drugs. This is consistent with the ex-vivo observations of Lyles et al (1983) who, using hydralazine, were able to inhibit completely SSAO from rat aorta, but not heart.

From these results, it would seem that procarbazine and monomethylhydrazine have retained the selectivity for SSAO over MAO activities which they displayed in-vitro (Holt et al 1992a, b). Following intraperitoneal administration of procarbazine to rats, it is metabolized rapidly by

Table 1. The effects of procarbazine and monomethylhydrazine on the metabolism of benzylamine perfused through the mesenteric arterial bed of the rat. Metabolite measurements are corrected by subtracting 3·73 nmol, the value obtained from a blank perfusion.

Drug treatment	Total radioactivity recovered (nmol)	Radioactivity due to metabolites (nmol)	Corrected metabolite levels (nmol)	Metabolites present (% of control value)
Control	244·6 (243·3–246·0)	42·3 (41·2–43·5)	38·6	100
Procarbazine	254·7 (249·7–259·7)	6·50 6·16–6·84)	2·77	7·2
Monomethylhydrazine	254·6 (253·7–255·6)	7·00 (6·8–7·2)	3·27	8·5

Data are the means of results from two experiments. The range is indicated in parentheses.

cytochrome P450 and MAO enzymes to azoprocarbazine (Dewald et al 1969; Coomes & Prough 1983; Prough et al 1984), a potent SSAO inhibitor (Holt et al 1992b). Thus, it is likely that this metabolite is responsible for much of the inhibition of SSAO measured in these studies following administration of procarbazine.

A simple explanation for the increased activity of MAO induced by procarbazine and monomethylhydrazine is not readily forthcoming. Rat liver MAO B activity was potentiated by monomethylhydrazine to approximately 140% of that in controls, in a manner which was not dose-dependent. MAO A activity in BAT was increased by both drugs in a dose-dependent fashion, although the potentiation was very much more pronounced with monomethylhydrazine. When the effects of monomethylhydrazine on BAT were examined more closely, results suggested that increased activity was not the result of a direct interaction between the drug and the enzyme since the same phenomenon was not apparent in-vitro. Increases in MAO activity occur physiologically in a number of situations (see Callingham 1984). However, this is generally as a result of increased enzyme synthesis and the effects seen here are too rapid to be accounted for in this way.

Potentiation ex-vivo of MAO A activity in rat BAT has been observed previously following acute administration of benserazide (Lyles & Callingham 1982). This effect was dose-dependent, the highest dose ($150\,mg\,kg^{-1}$) increasing enzyme activity to approximately 150% of that in controls. However, when benserazide was administered chronically for seven days, MAO A activity was not different from controls. These effects were not evident when homogenates of aorta or heart were examined. Such a rapid potentiation of MAO A produced by benserazide and by procarbazine and monomethylhydrazine, coupled with a recovery of enzyme activity to control levels despite continued administration of benserazide, may implicate the involvement of a second carbonyl-containing enzyme or receptor involved in regulation of MAO activity. We have observed previously that procarbazine displays some affinity for adrenergic receptors in rat blood vessels (A. Holt, unpublished results) and it may thus be of interest to determine whether ligands for the β_3-adrenergic receptor found in rat BAT can modulate MAO A activity in that tissue.

Blood vessel homogenates have been used extensively to examine the biochemical properties of SSAO enzymes (Lewinsohn et al 1978; Clarke et al 1982; Precious et al 1988; Elliott et al 1989c) and such studies have shown benzylamine to be the preferred substrate in most cases. Results from perfusion experiments illustrate that the metabolism of [^{14}C]benzylamine by rat vascular SSAO is inhibited substantially following pretreatment of animals with procarbazine or monomethylhydrazine, providing further support for the usefulness of these compounds in ex-vivo studies. A blank value, which accounts for background and also labelled contaminants soluble in organic solvent, can be obtained from in-vitro assays by including HCl in the incubation mixture. It is not possible to obtain a blank in this way for perfusion studies and so an alternative method was devised, whereby benzylamine was perfused through the apparatus in the absence of a mesentery. By this method, it was determined that 3·73 nmol of degradation products was present in the perfusate. When this figure was subtracted from the results obtained in the original experiments, the apparent degree of inhibition of SSAO following procarbazine or monomethylhydrazine had been increased substantially, such that intraperitoneal administration of these drugs had reduced metabolism of perfused benzylamine by a similar degree to that seen when the amine had instead been incubated with rat aortic homogenates ex-vivo. These results suggest that inclusion of a blank perfusion of amine substrate, without a mesentery, would provide an indication of the extent of the error produced by labelled contaminants and should perhaps be adopted as standard procedure in such perfusion experiments.

Although benzylamine is universally stated to be a synthetic SSAO substrate, it does occur naturally and can be isolated from the roots and bark of *Moringa pterygosperma* (Chakravarti 1955), a tropical, deciduous tree. However, benzylamine has not yet been detected in animal tissues and it seems unlikely that this aromatic amine is the natural substrate for SSAO. Nevertheless, deamination of any primary monoamine by vascular SSAO will result in the release of ammonia and H_2O_2 at the external cell surface and these molecules might modulate vascular tone. When included in the fluid perfusing the rat mesenteric arterial bed, benzylamine itself can cause a leftward shift in the dose-response curve to adrenaline (Elliott & Callingham 1991). This effect was a result of some action of the parent amine, since inhibition of SSAO increased the shift. Similarly, co-administration of benzylamine and carbachol increased the relaxing potency of the latter, an effect which was abolished by SSAO inhibition. It was suggested that increased availability of ammonia for nitric oxide (endothelial-derived relaxing factor; EDRF) synthesis might explain this observation (Moncada et al 1989). On the other hand, H_2O_2 can relax rabbit aorta precontracted by phenylephrine, but not by K^+, in a manner which is partly dependent on the presence of an intact endothelium, and can attenuate the contractile responses to phenylephrine, histamine and 5-HT (Iesaki et al 1994). H_2O_2 can also cause relaxation of bovine pulmonary arteries (Burke & Wolin 1987), perhaps through activation of adenylate cyclase (Wright & Drummond 1983; Haenen et al 1988). This too might augment the relaxant effects of EDRF released by carbachol. It is thus clear that some of the products of amine metabolism, as well as the amines themselves, may modulate vascular tone.

In conclusion, these experiments have provided evidence that administration of procarbazine or monomethylhydrazine to rats results in an ubiquitous inhibition of SSAO enzymes without concomitant inhibition of MAO activities. These compounds, and probably also azoprocarbazine, would seem suitable for use in- and ex-vivo, when selective inhibition of SSAO is required. However, it is important to remember that other enzymes, sensitive to inhibition by carbonyl reagents, might be affected, particularly when high doses of procarbazine are administered. The possibility of MAO potentiation must be considered when interpreting results from studies where procarbazine and related compounds are used in this manner. However, the ability of these drugs to potentiate MAO certainly merits further study since any endogenous system for regulating MAO activity might present a future target for new drug therapies.

Studies of pharmacological effects of SSAO inhibition in preparations like the perfused mesenteric arterial bed would seem currently to provide the greatest hope of a breakthrough in the search for the function of these enzymes. While benzylamine is a useful tool in such experiments, the perfusion of physiologically occurring amines such as methylamine, aminoacetone, tryptamine, phenylethylamine, or indeed histamine, which is a substrate for SSAO in the rat mesenteric bed (Buffoni et al 1994), might provide more relevant and meaningful data. The time now seems right for thorough examination of the effects of these amines, and of H_2O_2 and ammonia, on vascular tone and reactivity and of the role played by SSAO in modulating such effects in the mesenteric bed.

Acknowledgements

A. Holt was a Medical Research Council Scholar. We thank the Horserace Betting Levy Board for generous financial support and Dr. M. Da Prada of Hoffmann-La Roche for his donation of procarbazine.

References

Andree, T.H., Clarke, D.E. (1982) Characteristics and specificity of phenelzine and benserazide as inhibitors of benzylamine oxidase and monoamine oxidase. Biochem. Pharmacol. 5: 825–830

Bach, A. W. J., Lan, N. C., Johnson, D. L., Abell, C. W., Bembenek, M. E., Kwan, S.-W., Seeburg, P. H., Shih, J. C. (1988) cDNA cloning of human liver monoamine oxidase A and B: molecular basis of differences in enzymatic properties. Proc. Natl. Acad. Sci. USA 85: 4934–4938

Barrand, M. A., Callingham, B. A. (1982) Monoamine oxidase activities in brown adipose tissue of the rat: some properties and subcellular distribution. Biochem. Pharmacol. 31: 2177–2184

Barrand, M. A., Callingham, B. A. (1984) Solubilization and some properties of a semicarbazide-sensitive amine oxidase in brown adipose tissue of the rat. Biochem. J. 222: 467–475

Barrand, M. A., Callingham, B. A., Fox, S. A. (1984) 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

Buffoni, F., Banchelli, G., Ignesti, G., Pirisino, R., Raimondi, L. (1994) The role of semicarbazide-sensitive amine oxidase with a high affinity for benzylamine (Bz.SSAO) in the catabolism of histamine in the mesenteric arterial bed of the rat. Agents Actions 42: 1–6

Burke, T. M., Wolin, S. (1987) Hydrogen peroxide elicits pulmonary arterial relaxation and guanylate cyclase activation. Am. J. Physiol. 252: H721–H732

Callingham, B. A. (1984) Factors affecting cardiac MAO activity. In: Tipton, K. F., Dostert, P., Strolin Benedetti, M. (eds) Monoamine Oxidase and Disease. Prospects for Therapy with Reversible Inhibitors. Academic Press, London, pp 233–241

Callingham, B. A., Holt, A., Elliott, J. (1991) Properties and functions of the semicarbazide-sensitive amine oxidases. Biochem. Soc. Trans. 19: 228–233

Chakravarti, R. N. (1955) Chemical identity of moringine. Bull. Calcutta School Trop. Med. 3: 162–163

Clarke, D. E., Lyles, G. A., Callingham, B. A. (1982) A comparison of cardiac and vascular clorgyline-resistant amine oxidase and monoamine oxidase. Biochem. Pharmacol. 31: 27–35

Coomes, M. W., Prough, R. A. (1983) The mitochondrial metabolism of 1,2-disubstituted hydrazines, procarbazine and 1,2-dimethylhydrazine. Drug Metab. Disp. 11: 550–555

Coquil, J. F., Goridis, C., Mack, G., Neff, N. H. (1973) Monoamine oxidase in rat arteries: evidence for different forms and selective localization. Br. J. Pharmacol. 48: 590–599

Dewald, B., Baggiolini, M., Aebi, H. (1969) *N*-Demethylation of *p*-(N^1-methylhydrazino methyl)-*N*-isopropyl benzamide (procarbazine), a cytostatically active methylhydrazine derivative, in the intact rat and in the isolated perfused rat liver. Biochem. Pharmacol. 18: 2179–2186

Elliott, J., Callingham, B. A. (1991) Effect of benzylamine and its metabolites on the responses of the isolated perfused mesenteric arterial bed of the rat. J. Auton. Pharmacol. 11: 323–335

Elliott, J., Callingham, B. A., Sharman, D. F. (1989a) Metabolism of amines in the isolated perfused mesenteric arterial bed of the rat. Br. J. Pharmacol. 98: 507–514

Elliott, J., Callingham, B. A., Sharman, D. F. (1989b) The influence of amine metabolizing enzymes on the pharmacology of tyramine in the isolated perfused mesenteric arterial bed of the rat. Br. J. Pharmacol. 98: 515–522

Elliott, J., Callingham, B. A., Sharman, D. F. (1989c) Semicarbazide-sensitive amine oxidase of the rat aorta: interactions with naturally occurring amines and their structural analogues. Biochem. Pharmacol. 38: 1507–1515

Haenen, G. R., Van Dansik, P., Vermeulen, N. P., Timmerman, H., Bast, A. (1988) The effect of hydrogen peroxide on beta-adrenoceptor function in the heart. Free Radic. Res. Commun. 4: 243–249

Holt, A., Callingham, B. A. (1993) Pharmacological evidence that rat blood vessel smooth muscle semicarbazide-sensitive amine oxidase is an ectoenzyme. Br. J. Pharmacol. 108: 158P

Holt, A., Callingham, B. A. (1994a) Location of the active site of rat vascular semicarbazide-sensitive amine oxidase. J. Neural Transm. 41 (Suppl.): 433–437

Holt, A., Callingham, B. A. (1994b) The ex vivo effects of procarbazine and methylhydrazine on some rat amine oxidase activities. J. Neural Transm. 41 (Suppl.): 439–443

Holt, A., Sharman, D. F., Callingham, B. A., Kettler, R. (1992a) characteristics of procarbazine as an inhibitor in-vitro of rat semicarbazide-sensitive amine oxidase. J. Pharm. Pharmacol. 44: 487–493

Holt, A., Sharman, D. F., Callingham, B. A. (1992b) Effects in-vitro of procarbazine metabolites on some amine oxidase activities in the rat. J. Pharm. Pharmacol. 44: 494–499

Iesaki, T., Okada, T., Yamaguchi, H, Ochi, R. (1994) Inhibition of vasoactive amine induced contractions of vascular smooth muscle by hydrogen peroxide in rabbit aorta. Cardiovasc. Res. 28: 963–968

Klinman, J. P., Mu, D. (1994) Quinoenzymes in biology. Annu. Rev. Biochem. 63: 299–344

Lewinsohn, R. (1981) Amine oxidase in human blood vessels and non-vascular smooth muscle. J. Pharm. Pharmacol. 33: 569–575

Lewinsohn, R. (1984) Mammalian monoamine-oxidizing enzymes, with special reference to benzylamine oxidase in human tissues. Brazil. J. Med. Biol. Res. 17: 223–256

Lewinsohn, R., Böhm, K. H., Glover, V., Sandler, M. (1978) A benzylamine oxidase distinct from monoamine oxidase B–widespread distribution in man and rat. Biochem. Pharmacol. 27: 1857–1863

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275

Lyles, G. A. (1984) The interaction of semicarbazide-sensitive amine oxidase with MAO inhibitors. In: Tipton, K. F., Dostert, P., Strolin Benedetti, M. (eds) Monoamine Oxidase and Disease. Prospects for Therapy with Reversible Inhibitors. Academic Press, London, pp 547–556

Lyles, G. A. (1994) Properties of mammalian tissue-bound semicarbazide-sensitive amine oxidase: possible clues to its physiological function? J. Neural Transm. 41 (Suppl.): 387–396

Lyles, G. A., Callingham, B. A. (1982) In vitro and in vivo inhibition by benserazide of clorgyline-resistant amine oxidases in rat cardiovascular tissues. Biochem. Pharmacol. 31: 1417–1424

Lyles, G. A., Chalmers, J. (1992) The metabolism of aminoacetone to methylglyoxal by semicarbazide-sensitive amine oxidase in human umbilical artery. Biochem. Pharmacol. 43: 1409–1414

Lyles, G. A., Garcia-Rodriguez, J., Callingham, B. A. (1983) Inhibitory actions of hydralazine upon monoamine oxidizing enzymes in the rat. Biochem. Pharmacol. 32: 2515–2521

Lyles, G. A., Holt, A., Marshall, C. M. S. (1990) Further studies on the metabolism of methylamine by semicarbazide-sensitive amine

oxidase activities in human plasma, umbilical artery and rat aorta. J. Pharm. Pharmacol. 42: 332–338

Matsuzaki, R., Fukui, T., Sato, H., Ozaki, Y., Tanizawa, K. (1994) Generation of the topa quinone cofactor in bacterial monoamine oxidase by cupric ion-dependent autooxidation of a specific tyrosyl residue. FEBS Lett. 351: 360–364

McGregor, D. D. (1965) The effect of sympathetic nerve stimulation on vasoconstrictor responses in perfused mesenteric blood vessels of the rat. J. Physiol. 177: 21–30

Moncada, S., Palmer, R. M. J., Higgs, E. A. (1989) Biosynthesis of nitric oxide from L-arginine. A pathway for the regulation of cell function and communication. Biochem. Pharmacol. 38: 1709–1715

Nakos, G., Gossrau, R. (1994) Light microscopic visualisation of semicarbazide-sensitive amine oxidase (benzylamine oxidase) using a cerium method. Folia Histochem. Cytobiol. 32: 3–10

Precious, E., Gunn, C. E., Lyles, G. A. (1988) Deamination of methylamine by semicarbazide-sensitive amine oxidase in human umbilical artery and rat aorta. Biochem. Pharmacol. 37: 707–713

Prough, R. A., Brown M. I., Dannan, G. A., Guengerich, F. P. (1984) Major isozymes of rat liver microsomal cytochrome P450 involved in the *N*-oxidation of *N*-isopropyl-α-(2-methylazo)-*p*-toluamide, the azo derivative of procarbazine. Cancer Res. 44: 543–548

Raimondi, L., Pirisino, R., Ignesti, G., Capecchi, S., Banchelli, G., Buffoni, F. (1991) Semicarbazide-sensitive amine oxidase activity (SSAO) of rat epididymal white adipose tissue. Biochem. Pharmacol. 41: 467–470

Wibo, M., Duong, A. T., Godfraind, T. (1980) Subcellular location of semicarbazide-sensitive amine oxidase in rat aorta. Eur. J. Biochem. 112: 87–94

Wright, M., Drummond, G. I. (1983) Inactivation of the β-adrenergic receptor in skeletal muscle by dithiols. Biochem. Pharmacol. 32: 509–515