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AMINE OXIDASE ACTIVITIES IN RAT BREAST CANCER INDUCED EXPERIMENTALLY WITH 7,12-DIMETHYLBENZ(α)ANTHRACENE

JOSÉ M. LIZCANO,* EDUARD ESCRICH,‡ TERESA RIBALTA,§ JAUME MUNTANÉ§ and MERCEDES UNZETA*

*Departament de Bioquímica i Biologia Molecular and ‡Departament de Biologia Cellular i Fisiologia, Facultat de Medicina de la Universitat Autonama de Barcelona and §Hospital Clinic Provincial de Barcelona, Departament de Anatomia Patològica, Facultat de Medicina, Universitat de Barcelona, Barcelona, Spain

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Abstract—The activities and distribution of monoamine oxidase (MAO) and semicarbazide-sensitive amine oxidase (SSAO) in solid breast tumour induced in the rat by treatment with 7,12-dimethylbenz(α)anthracene (DMBA) were studied. The mammary tumours were classified according to anatomopathological criteria into: the benign fibroadenoma (FAD) and the malignant adenocarcinoma (ADC) and infiltrant adenocarcinoma (I-ADC). The proportions of total MAO (15%) and SSAO activities (85%) did not change with malignancy. However, an increasing degree of malignancy was associated with an increase in MAO-A activity and a decrease in MAO-B and SSAO activities. Kinetic constants were calculated for SSAO and for each MAO form separately, using specific substrates. The K_m values did not change significantly with the degree of malignancy, but V_{max} values for MAO-A increased whereas V_{max} for SSAO and MAO-B diminished with malignancy. The dependence of SSAO activity on protein concentration indicated the presence of endogenous reversible inhibitory material in extracts from the more malign tumours. This inhibitor was associated with the microsomal fraction and was not removed by dialysis. It was also present in detergent-solubilized extracts, suggesting that the phenomenon might be due to an association of the enzyme itself producing an inactive species.

Monoamine oxidase [amine: oxygen oxidoreductase (deaminating) (flavin-containing) EC 1.4.3.4] (MAO) is localized mainly in the mitochondrial outer membrane and is widely distributed in animal tissues. It exists in two forms which differ in their substrate specificities and sensitivity to inhibitors. MAO-A is sensitive to inhibition by nanomolar concentrations of the acetylenic compound clorgyline [1] and is active towards 5-hydroxytryptamine (5-HT), whereas MAO-B is sensitive to inhibition by nanomolar concentrations of (−)-deprenyl [2] and is active towards benzylamine (BZ) and 2-phenylethylamine (PEA) as substrates.

Another amine oxidase activity that is present in relatively large amounts in vascularized tissues has been described [3]. This enzyme is resistant to inhibition by acetylenic compounds such as deprenyl and clorgyline, but is sensitive to inhibition by carbonyl reagents such as semicarbazide and consequently it has been named semicarbazide-sensitive amine oxidase (SSAO) [amine: oxygen oxidoreductase (deaminating) (copper-containing) [EC 1.4.3.6]. Because benzylamine (a non-physiological amine) is a particularly good substrate for this enzyme, it has sometimes been termed benzylamine oxidase. However, the natural substrates have yet to be identified and thus the physiological role of the enzyme is still unclear (see Ref. 4 for review). Its presence in highly vascularized tissues suggests that it may be involved in angiogenic processes in normal or pathological tissues. The EC 1.4.3.6 class contains several different amine oxidases including diamine oxidase, polyamine oxidase, tissue SSAO and serum benzylamine oxidase. These enzymes have a number of common properties, but differ in several respects. For example, both serum benzylamine oxidase and SSAO oxidize benzylamine although the former is a soluble enzyme and SSAO is membrane bound.

Polyamines have been shown to regulate cell division of cells grown in culture [5, 6]. The concentration of putrescine in human fibroblasts has been shown to be critical for enhancement of proliferation, and inhibition of cell growth occurs at higher concentrations [5]. This growth-inhibitory action of polyamines has also been demonstrated in cultures of mammalian lymphocytes [6, 7] and it has been suggested that it is caused by the toxic products of polyamine oxidation. The enzyme responsible for this oxidation has been identified as benzylamine oxidase, and its activity has been reported [8] to be altered in patients with malignant tumours.

The oxidation of amine by MAO on SSAO gives rise to H_2O_2 and aldehydes. Recent studies on the mechanism of cancer regulation in the embryo [9] showed that these reaction products, resulting from the metabolism of polyamines by serum benzylamine oxidase, caused programmed cell death in the embryo and also killed cancer cells [10].

Alterations in monoamine content in different kinds of tumours has been reported [11]. Cancer patients have significantly raised MAO activities in platelets [12] and consequently there is an alteration

† Correspondence should be addressed to: José M. Lizcano, Departament de Bioquímica i Biologia Molecular, Facultat de Medicina, Universitat Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.

of monoamine content during the metastasic process. Growth of both chemically induced and transplanted tumours in rat was accompanied by an increase of GABA in the hypothalamus and hippocampus and a reduced concentration of monoamines and their metabolites in the hypothalamus and the caudate nucleus [13]. This alteration in amine content correlated with the metastasic processes which might imply alterations of amine oxidase activities.

So far, research on amine oxidase in cancer has focused on the alteration in serum benzylamine oxidase, polyamine oxidase or diamine oxidase content; there have been no detailed studies on SSAO and MAO activities in solid tumours. In the present study we have examined changes in activity and kinetic parameters of SSAO and both forms of MAO in chemically-induced breast tumours in the rat. In order to avoid the variability and irreproducibility often associated with experimental data obtained with human tissue we selected an experimental model in which breast tumours of different degrees of malignancy were induced in rats with 7,12-dimethylbenz(α)anthracene (DMBA).

MATERIALS AND METHODS

Experimental induction of mammary cancer. Mammary cancer was induced in 52-day-old female Sprague–Dawley rats weighing 160–180 g by a single dose per animal of 1 mL sesame oil containing 20 mg 7,12-dimethylbenz(α)anthracene (DMBA) administered by gastric instillation [14, 15]. Prior to instillation the rats were semianesthetized with 5 mg of ketamine hydrochloride. The validity of this experimental model has been broadly confirmed [16] and it was selected because of the close similarity of the induced tumours to those in humans. Rats were fed *ad lib.* on an A04 standard diet.

The histological diagnosis of experimental mammary tumours was based on Young and Hallowey's criteria [17]: the tumours were classified by anatomopathological criteria as fibroadenoma (FAD), the benign one, and the more malignant adenocarcinoma (ADC) and infiltrant adenocarcinoma (I-ADC).

Tumours were dissected and small aliquots separated for anatomopathological diagnosis. Samples maintained in liquid N_2 were disrupted mechanically, homogenized in 50 mM potassium phosphate buffer, pH 7.2 (1:10 w/v), and stored in aliquots at −80°. Before each assay, the preparations were disaggregated by sonication at low frequency for 20 sec. Comparisons with fresh tissue homogenates showed that the manipulation had no significant effect on the enzyme activities.

Protein was determined by a modification of the Lowry method [18].

MAO and SSAO assay. Monoamine oxidase activity was determined radiochemically at 37° by a modification [19] of the method of Otsuka and Kobayashi [20]. 2-Phenylethylamine (PEA; [ethyl-1-^{14}C]phenylethylamine hydrochloride; New England Nuclear, Boston, MA, U.S.A.), benzylamine (BZ; [7-^{14}C]benzylamine hydrochloride; Amersham International, Amersham, U.K.), 5-hydroxytryptamine (5-HT; 5-hydroxy[side-chain-2-^{14}C] tryptamine creatinine sulphate; Amersham International) and tyramine (TYR; tyramine(*p*-hydroxyphenyl[2-^{14}C]ethylamine hydrochloride; Amersham International) were used as substrates. Assay mixtures contained a final volume of 225 μL of 50 mM potassium phosphate buffer (pH 7.2), substrate and 200 μg of protein. The reaction was stopped by adding 100 μL of 2 M citric acid and the products were extracted into 1:1 (v/v) toluene/ethyl acetate containing 0.6% (w/v) PPO. The radioactivity of the extract was determined by liquid scintillation counting. SSAO activity towards benzylamine (1 μM) a substrate was determined using 200 μg of protein at which concentration there was no significant deviation from a linear dependence of initial velocity on enzyme concentration. The reaction was stopped by adding 100 μL of 2 M citric acid and the products were extracted as before.

Subcellular distribution of MAO and SSAO activities. I-ADC-type tumours were homogenized in 50 mM potassium phosphate buffer, pH 7.2, containing 0.25 M sucrose and centrifuged at 600 *g* for 10 min to obtain the nuclear pellet. The supernatant was centrifuged at 8000 *g* for 10 min to give a crude mitochondrial fraction and at 200,000 *g* for 30 min to give the microsomal fraction and the supernatant, cytosolic fraction.

Solubilization of SSAO activity by the detergent β-octyl-glucoside. Microsomal pellets from I-ADC tissue containing 3 mg/mL of protein were added to different concentrations of β-octyl-glucoside (OG) to give final detergent concentrations of 0.5, 0.75, 1 and 1.5% (w/v) in 50 mM potassium phosphate buffer, pH 7.2. After 30 min shaking at 4° the mixtures were centrifuged at 100,000 *g* for 60 min in a Beckman SW-60 Ti rotor and the distributions of protein and SSAO activity towards 16 μM benzylamine were determined in the corresponding pellets and supernatants.

RESULTS

Specific activities

The linearity of the amount of product formed with time was checked for each assay and it was shown to be maintained for 6, 15, 20 and 60 min with PEA (20 μM), TYR (100 μM), 5-HT (100 μM) and BZ (1 μM) as substrate, respectively. The linearity of initial velocity with the amount of protein was also determined in each case.

Specific activities were determined for MAO-A and MAO-B towards 5-HT and PEA as substrate, respectively. After inhibition with semicarbazide (1 mM) or clorgyline (1 mM), the remaining activity was measured towards BZ (1 μM) in order to determine the specific activities of MAO and SSAO towards this substrate. Assays were carried out on individual tumours and on pooled material from several tumours with the same histological characteristics.

The results obtained are shown in Table 1. There was no significant difference between the specific activities obtained with single and pooled tumours for the same tumour type. Consequently, due to the

Table 1. Specific activities of SSAO and MAO enzyme present in different kinds of breast tumour

		FAD	ADC	I-ADC
5-HT	I	124.84 ± 15.85	289.77 ± 54.17	324.5 ± 29.33
	P	147.24 ± 0.50	253.84 ± 16.57	326.7 ± 10.46
PEA	I	115.48 ± 18.82	91.39 ± 29.36	61.20 ± 11.13
	P	145.99 ± 2.41	82.24 ± 2.08	61.20 ± 0.82
BZ (MAO + SSAO)	I	21.88 ± 9.22	6.21 ± 1.86	2.25 ± 0.85
	P	25.37 ± 1.20	6.46 ± 0.31	4.18 ± 0.23
BZ (MAO)	I	3.09 ± 0.94	0.91 ± 0.30	1.22 ± 0.38
	P	3.79 ± 0.67	0.87 ± 0.09	0.63 ± 0.26
BZ (SSAO)	I	18.72 ± 8.33	4.78 ± 1.78	2.38 ± 1.27
	P	17.98 ± 1.89	3.47 ± 0.37	2.59 ± 0.12

Specific activities were expressed in pmol/min·mg protein. In each case the activity not desired had been inhibited previously by the corresponding specific inhibitor. Enzyme activities are mean values ± SE of nine individual tumors (I) corresponding to each histopathological type. The tumours pooled (P) were prepared from eight tumours of the same histopathological type and each value is the mean ± SE of three determinations.

small size of the individual tumours it was decided to carry out the remainder of the study with pooled tumour samples of the same anatomopathological type.

In all samples, the activity towards 5-HT, a specific substrate of MAO-A, was higher than that towards the MAO-B substrate PEA. The lowest specific activity was found with BZ as substrate. MAO-A Activity increased with malignancy, being some 2.5 times higher in I-ADC than in FAD. In contrast, MAO-B activity diminished with malignancy, I-ADC having only about half the specific activity found in FAD.

The total activity (MAO + SSAO) towards BZ (1 μM) decreased with malignancy, being about 10 times lower in I-ADC than in FAD. The activity of MAO towards BZ (1 μM) was considerably lower than that of SSAO. In all cases, specific activity towards BZ in benign tumours was higher than in malignant tumours.

The possible presence of plasma benzylamine oxidase in these samples was investigated by incubation of the tissue preparations for 30 min at 37° with different KCN concentrations (10^{-3}–10^{-10} M) before assay. KCN is a specific inhibitor of plasma benzylamine oxidase [21]. However, in none of the samples was activity towards BZ (1 μM) significantly inhibited.

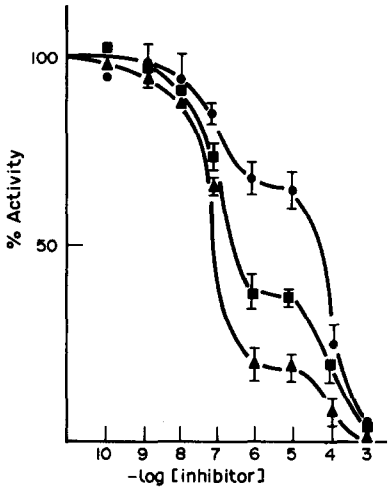

Fig. 1. Inhibition curves by clorgyline of MAO activity present in different kinds of breast tumours, measuring the activity towards TYR (100 μM) as substrate. I-ADC (▲). ADC (■) and FAD (●) were preincubated for 30 min at 37° with different inhibitor concentrations (10^{-10}–10^{-3} M). The remaining activity is expressed as a percentage of the control activity determined in the absence of inhibitor. Each value is the mean ± SE of three determinations.

Sensitivity to clorgyline

The inhibitory effect of clorgyline was studied with the different kinds of tumour preparation. Samples were incubated with the inhibitor in the concentration range 10^{-3}–10^{-10} M for 30 min at 37° before measuring the activity remaining towards TYR (100 μM) as common substrate of both MAO forms (Fig. 1). A double sigmoidal curve was obtained in all cases but the height of the plateau region varied. This indicates the presence of both forms of MAO in each type of tumour [1] but in different relative amounts. The proportion of MAO-A was higher in ADC and I-ADC (the more malignant one), and less in FAD (the most benign tumour). The percentage of each form of MAO present and the ratio MAO-A/MAO-B taken from these curves are summarized in Table 2.

Presence of semicarbazide-sensitive amine oxidase

The presence of SSAO activity was investigated by studying the inhibitory effect of semicarbazide. Samples were incubated with this compound in the concentration range 10^{-3}–10^{-10} M for 30 min at 37° before the activity remaining towards BZ (1 μM) was measured.

Studies on the effect of inhibitors on activities in the tumour samples allowed the contributions of the

Table 2. Distribution of each form of MAO and SSAO activity and MAO-A/MAO-B ratio present in different kinds of breast tumours

	FAD	ADC	I-ADC
% MAO-A*	31.6 ± 2.0	61.1 ± 0.7	80.8 ± 0.5
% MAO-B*	68.4 ± 2.0	38.9 ± 0.7	19.2 ± 0.5
MAO-A/MAO-B*	0.46 ± 0.06	1.57 ± 0.05	4.21 ± 0.10
MAO-A/MAO-B†	1.08 ± 0.02	3.17 ± 0.24	5.30 ± 0.28
% MAO‡	13.3 ± 6.4	14.3 ± 4.6	13.0 ± 5.2
% SSAO‡	86.4 ± 3.5	85.1 ± 5.9	85.6 ± 6.0

* Values (activity as % of control value) taken from the inhibition curves with clorgyline measuring the remaining activity towards TYR (100 μM) as substrate (Fig. 1). Each value is the mean ± SE of three determinations.

† Values obtained from MAO-A and MAO-B specific activities determined towards 5-HT (100 μM) and PEA (20 μM), respectively. Each value is the mean ± SE of nine individual tumours.

‡ Values (activity as % of control value) calculated from the inhibition curves with semicarbazide measuring the remaining activity towards BZ (1 μM) as substrate. Each value is the mean ± SE of three determinations.

Table 3. Subcellular distribution of MAO and SSAO activities in ADC tumours

	MAO (TYR)	MAO (BZ)	SSAO (BZ)
Nuclear	317.6 ± 13.5	3.56 ± 0.47	1.91 ± 0.45
Mitochondria	960.8 ± 18.6	7.83 ± 0.33	5.22 ± 0.23
Microsomes	203.3 ± 8.7	0.35 ± 0.09	6.67 ± 0.35
Cytosol	—	—	—

Specific activities were determined towards 100 μM TYR and 1 μM BZ as substrate and were expressed in pmol/min·mg protein. Samples were inhibited prior to assay with semicarbazide (1 mM) or clorgyline (1 mM) in order to measure MAO and SSAO towards BZ as substrate, respectively. Each value is the mean ± SE of three determinations.

different amine oxidases to be determined. At 10^{-3} M semicarbazide the activity remaining corresponds to MAO, which is insensitive to carbonyl reagents, and the inhibited activity corresponds to SSAO [3]. The percentages of each type of activity determined are summarized in Table 2. MAO activity represented some 15% and SSAO activity some 85% of the total in each type of tumour. Similar values were obtained from studies on inhibition by clorgyline (data not shown).

Subcellular distribution of MAO and SSAO activities

The subcellular distribution of the MAO and SSAO activities present in mammary tumours are shown in Table 3. MAO activity is located mainly in the crude mitochondrial fraction in agreement with results of studies using other tissues [1, 2] whereas SSAO appears to be distributed rather evenly between the mitochondrial and microsomal fractions. As can be seen from this table, the mitochondrial activity towards BZ (1 μM) is due mainly to MAO (61%) with SSAO contributing some 39%, whereas in microsomes the activity is almost entirely due to SSAO (95%) with about 5% of the total being contributed by MAO. Barrand and Callingham [22] showed also that the microsomal fraction from brown adipose tissue was rich in SSAO. They concluded from further studies that much of the enzyme activity was in fact associated with the plasma membrane. Unfortunately, insufficient material was available in the present studies to show whether a similar location accounts for the apparent microsomal fraction of the enzyme from mammary tumours. Because of the subcellular distribution of SSAO the microsomal fraction was used to solubilize SSAO activity. The possibility that the SSAO activity associated with the mitochondrial fraction may arise from contamination of that fraction with microsomal material cannot be completely excluded. However, extensive washing of this pellet with the same buffer used in the subcellular distribution studies followed by centrifugation and resuspension four times failed to reduce this mitochondrial SSAO activity significantly.

Determination of kinetic parameters

K_m and V_{max} values were estimated for each activity from Lineweaver–Burk plots. 5-HT was used to measure kinetic constants for MAO-A after inhibition of MAO-B by preincubation for 30 min at 37° with 10^{-7} M deprenyl. PEA was used to determine kinetic constants for MAO-B in samples that had been preincubated with 10^{-7} M clorgyline for 30 min at 37° to inhibit MAO-A activity. Kinetic constants for SSAO were measuring towards BZ as substrate after inhibition of the total MAO activity

Table 4. Kinetic parameters of SSAO and MAO activity in different types of breast tumours

		FAD	ADC	I-ADC
5-HT (MAO-A)	K_m	121.1 ± 4.2	111.3 ± 7.9	98.5 ± 3.6
	V_{max}	333.1 ± 3.3	551.6 ± 9.9	636.4 ± 6.2
PEA (MAO-B)	K_m	13.9 ± 2.7	9.5 ± 0.5	9.5 ± 0.5
	V_{max}	270.6 ± 9.5	123.6 ± 3.8	91.9 ± 1.7
BZ (SSAO)	K_m	7.2 ± 0.6	8.7 ± 0.6	4.9 ± 0.7
	V_{max}	157.9 ± 3.3	37.7 ± 0.7	16.4 ± 0.5

MAO-A was assayed using five different concentrations of 5-HT (50, 100, 300, 500 and 1000 μM) after inhibition of MAO-B with deprenyl (10^{-7} M). MAO-B was assayed using five different concentrations of PEA (5, 10, 20, 30 and 50 μM) after inhibition of MAO-A with clorgyline (10^{-7} M). Kinetic constants of SSAO activity were determined towards five different concentrations of BZ (1, 4, 16, 32 and 64 μM) after inhibition of MAO with clorgyline (1 mM). Kinetic constants were expressed in μM (K_m) and pmol/min · mg (V_{max}). Correlation coefficients of each linear regression of Lineweaver–Burk plots were higher than 0.999. Each value is the mean ± SE of three determinations.

by incubation with 10^{-3} M clorgyline for 30 min at 37°. The results are summarized in Table 4.

It can be seen that MAO-B and SSAO had considerably lower K_m values towards their respective specific substrates than had MAO-A. The K_m values of each enzyme from the tumour samples of different degrees of malignancy were similar. In the case of MAO-A V_{max} values were higher in I-ADC and lower in FAD with ADC samples showing intermediate values. In contrast, V_{max} values of MAO-B were higher in the benign tumour (FAD) and lower in I-ADC. The latter correlation was also observed for SSAO activity. These results could be explained in terms of a higher molecular concentration of MAO-A in the most malignant tumours compared to the benign ones and a higher molecular concentration of MAO-B and SSAO in the most benign tumours, relative to the malign ones.

Presence of endogenous inhibition

The possible presence of endogenous inhibitors or activators was studied by measuring the dependence of the activity on protein concentration (0–600 μg protein). BZ was used as substrate for SSAO activity and TYR as substrate for MAO activity. In the case of MAO activity no loss of linearity with different enzyme concentrations was observed, with either MAO-A or MAO-B.

The dependence of SSAO activity on protein concentration, assayed with either 16 or 1 μM benzylamine as substrate, showed a downward curvature which would be consistent with the presence of an endogenous inhibitor in the preparation [23]. Such behaviour was detected in both ADC and I-ADC malignant tumours but not in the FAD samples (Fig. 2). These results might explain the decrease of V_{max} values for SSAO activity shown in Table 4.

Study of the nature of this endogenous inhibition

Samples of ADC and I-ADC (containing 400 μg protein) were dialysed overnight against 50 mM potassium phosphate buffer, pH 7.2. SSAO activity

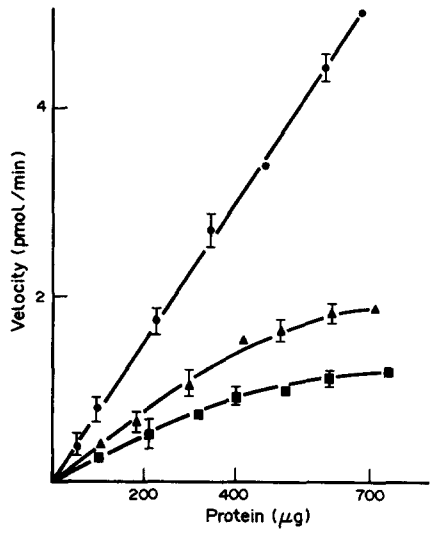

Fig. 2. Linearity of different SSAO concentrations (0–700 μg) using I-ADC (■), ADC (▲) and FAD (●) as enzyme samples. Velocity was measured with BZ (1 μM) as substrate at 37° for 30 min. Each point is the mean ± SE of four determinations.

was assayed before and after dialysis. The results obtained showed no change in the activity or in the non-linear dependence on protein concentration (data not shown). This indicates that the possible endogenous inhibitor, present only in malignant tumours, was non-dialysable. Thus its molecular weight would be greater than about 5000. Alternatively, the downward-curving enzyme concentration curve might have been due to a polymerizing system in which the dissociated form of the enzyme was the active species [23]. In this case, dialysis would not have been expected to remove the cause of non-linearity.

Subcellular fractionation was performed in order to isolate the microsomal fraction from the malignant tumours. This showed a similar non-linear

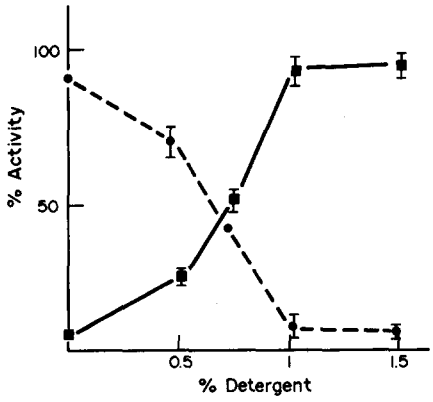

Fig. 3. Solubilization of SSAO from I-ADC tumours. Samples of microsome pellet containing 3 mg/mL of protein were treated at 4° with 0.5, 0.75, 1 and 1% (w/v) β-octylglucoside (OG) in 50 mM potassium phosphate buffer, pH 7.2, and centrifuged at 100,000 *g* for 1 hr. Supernatant (■) and pellet (●) samples were assayed for SSAO activity with BZ (16 μM) as substrate. Each point is the mean ± SE of three determinations.

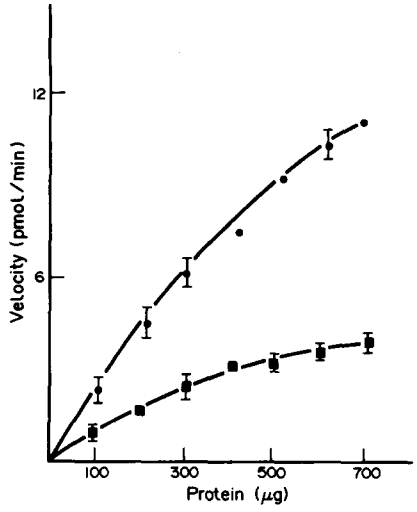

Fig. 4. Linearity of different SSAO concentrations (0–700 μg) using (●) solubilized I-ADC with 1.25%. β-octylglucoside and non-solubilized I-ADC (■). Velocity was measured with BZ (16 μM) as substrate at 37° for 30 min. Each point is the mean ± SE of four determinations.

dependence of activity on protein concentration to that observed with the disrupted preparations, indicating that the possible endogenous inhibitor was bound to the SSAO activity in the microsomal fraction.

To investigate whether this effect on SSAO activity in the more malignant tumours was due to changes that are known to occur in membrane composition in malignancy [24, 25] samples were rendered soluble by treatment with OG. The most effective OG concentrations for solubilizing SSAO activity were found to be between 1 and 1.5% (Fig. 3). However, treatment with 1.25% OG did not affect the non-linear dependence of SSAO activity on protein concentration (Fig. 4).

DISCUSSION

Lewinsohn [8] reported an alteration in benzylamine oxidase activity in the serum of burns patients and those suffering from different types of tumours. Differences in diamine oxidase and monoamine oxidase activities in foregut and midgut carcinoid tumours in humans have also been reported [11]. In those studies the MAO activity was measured towards tryptamine, which is a common substrate of both forms of the enzyme. Although diamine oxidase activity has been reported [26] to alter with malignancy in the human brain there have been no previous studies on the behaviour of SSAO and the two forms of MAO in solid metastasic tissues in relation to the degree of malignancy.

In this study, it was found that the ratio of MAO-A/MAO-B increased significantly with malignancy. MAO-A activity increased with malignancy whereas MAO-B activity diminished. This phenomenon has also been observed [27] in cases of thyroid disease such as malignant goiter but the reported results were not statistically significant. The changes in the specific activities of both forms of MAO in breast tumours could be due, in part, to hormonal effects. Progesterone injection in rats has been reported to cause large and significant increases in 5-HT-oxidizing activity in the ovary and uterus, with the activity of MAO-B unaltered [28]. The involvement of such hormones in mammary pathology as well as in normal mammary gland function has been reported [29]. Thus, the significant increase of breast tumour MAO-A activity with malignancy might be due to hormonal effects. However, further studies on the variation of the MAO-A/MAO-B ratio in non-hormone dependent tumours would be necessary in order to investigate this hypothesis further. An alternative possibility is that tumour transformation might involve a genetic response resulting in suppression of MAO-B and increased MAO-A synthesis. The physiological effects of increased MAO-A activity in the metastasic process remain to be clarified.

The advantage of this experimental model, the validity of which has been demonstrated [16], is that the variability and irreproducibility of results obtained with human tissue is avoided. In the present study, values obtained with individual tumours were not different from those obtained with tumours pooled within the same anatomopathological class.

The unchanged K_m values for each enzyme, the increase of V_{max} for MAO-A and the decrease of the maximum velocities of MAO-B and SSAO with malignancy would be consistent with changes in the quantities of these enzymes present. However, the non-linear dependence of SSAO activity on protein concentration in the more malignant samples would indicate the presence of an endogenous, reversible inhibitory factor. Such an inhibitor would have to be noncompetitive because of the unchanged K_m values. Since this factor was not removed by dialysis,

is bound to the insoluble microsomal fraction and was unaffected by solubilization with β-octylglucoside, the possibility that it might be an intrinsic property of the enzyme from malignant tissues cannot be excluded. Such a mechanism observed would imply the elaboration of a form of the enzyme in malignant tissues with an activity dependent on its polymerization state [23]. A similar phenomenon has been reported by Banchelli *et al.* [30] in the C3H strain of mouse kidney, but in this case the inhibitor was removed by dialysis.

The formation of new capillary vessels is a common event in several physiological and pathological situations such as tumour angiogenesis [31] and thus, the high proportion of SSAO enzyme in breast tumours could be related to such a vascularization process. The significance of the endogenous inhibitor in the more malignant samples remains to be established. Further comparative studies on the nature of the SSAO enzymes in samples of differing degrees of malignancy would be necessary to clarify the situation.

REFERENCES

1. Johnston JP, Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol* **17**: 1285–1297, 1968.
2. Knoll J and Magyar K, Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv Biochem Psychopharmacol* **5**: 393–408, 1972.
3. Lyles GA and Callingham BA, Evidence for a clorgyline-resistant monoamine metabolizing activity in the rat heart. *J Pharm Pharmacol* **27**: 682–691, 1975.
4. Callingham BA and Barrand MA, Some properties of semicarbazide-sensitive amine oxidase. *J Neural Transm* (Suppl) **23**: 37–54, 1987.
5. Pohjanpelto P and Rainald A, Identification of a growth-factor produced by human fibroblast "*in vitro*" as putrescine. *Nature (London) New Biol* **235**: 247–249, 1972.
6. Allen JC, Smith CJ and Curry MC, Identification of a thymic inhibitor ("chalone") of a lymphocyte transformation as a spermine complex. *Nature* **267**: 623–625, 1977.
7. Byrd WJ, Jacobs DM and Amoss MS, Synthetic polyamines added to cultures containing bovine sera reversibly inhibit "*in vitro*" parameters of immunity. *Nature* **267**: 621–623, 1977.
8. Lewinsohn R, Benzylamine oxidase. A marker of gynaecological cancer? *Pharmacol Toxicol* **60** (Suppl 1): 32, 1987.
9. Parchment RE, Lewellyn AL, Swartzendruber D and Pierce GB, Serum amine oxidase activity contributes to crisis in mouse embryo cell lines. *Proc Natl Acad Sci USA* **87**: 4340–4344, 1990.
10. Pierce GB, Lewellyn AL and Parchment RE, Mechanism of programmed cell death in the blastocyst. *Proc Natl Acad Sci USA* **86**: 3654–3658, 1989.
11. Feldman JM, Monoamine and diamine oxidase activity in the diagnosis of carcinoid tumors. *Cancer* **56**: 2855–2860, 1985.
12. Prasad A, Rampling RP, Glover V and Sandler M. Psychiatric morbidity and platelet monoamine oxidase activity in cancer patients. *Psychiatry Res* **22**: 111–116, 1987.
13. von Metzler A and Nitsch G, Carcinogenesis and the central nervous system. *Cancer Detect Prev* **9**: 259–277, 1986.
14. Huggins C, Morii S and Grand LC, Mammary cancer induced by a single dose of polynuclear hydrocarbons. *Am J Surg* **154**: 315–318, 1961.
15. Escrich E, Mammary cancer model induced by dimethylbenz(*a*)anthracene: a good experimental tool for the study of tumour markers. *Int J Biol Markers* **2**: 109–119, 1987.
16. Escrich E, Validity of the DMBA-induced mammary cancer model for the study of human breast cancer. *Int J Biol Markers* **2**: 197–206, 1987.
17. Young S and Hallowey RC, In: *Pathology of Tumours in Laboratory Animals* (Ed. Turosov), Vol. I, pp. 31–74. IARC, Lyon, 1973.
18. Hartree EF, Determination of protein: a modification of the Lowry method that gives a linear photometric response. *Anal Biochem* **40**: 442–427, 1972.
19. Fowler CJ and Tipton KF, Concentration dependence of the oxidation by the two forms of rat liver mitochondrial monoamine oxidase. *Biochem Pharmacol* **30**: 3329–3332, 1981.
20. Otsuka S and Kobayashi Y, A radioisotopic assay for monoamine oxidase determinations in human plasma. *Biochem Pharmacol* **13**: 995–1006, 1964.
21. Buffoni F and Della Corte L, Pig plasma benzylamine oxidase. *Adv Biochem Psychopharmacol* **5**: 133–149, 1972.
22. Barrand MA and Callingham BA, Monoamine oxidase activities in brown adipose tissue of the rat: some properties and subcellular distribution. *Biochem Pharmacol* **31**: 2177–2184, 1982.
23. Dixon M and Webb EC, *Enzymes*, 3rd edn. Longman, London, 1979.
24. Mountford CE and Wright LC, Organization of lipids in the plasma membranes of malignant and stimulated cells: a new model. *TIBS* **13**: 172–177, 1988.
25. Shinitzky M, Membrane fluidity in malignancy adversative and recuperative. *Biochim Biophys Acta* **738**: 251–261, 1984.
26. Mondovi B, Riccio P, Riccio A and Marcozzi GS, Amine oxidase activity in malignant human brain tumours. *Adv Polyamine Res* **4**: 183–191, 1983.
27. Hidaka N, Nagatsu I, Nagasaka A and Ishizuki Y, Monoamine oxidase activity of the thyroid glands in thyroid disease. *Clinica Chimica Acta* **23**: 383–385, 1969.
28. Mazunder RC, Glover V and Sandler M, Progesterone provokes a selective rise of monoamine oxidase A in the female genital tract. *Biochem Pharmacol* **29**: 1959, 1980.
29. Huggins CH, Briziarelly G and Sutton H, Rapid induction of mammary carcinoma in the rat and the influence of hormones on the tumors. *J Exp Met* **109**: 25–42, 1959.
30. Banchelli G, Buffoni F and Raimondi I, On the presence of a clorgyline resistant benzylamine oxidase activity in mouse kidney. *Pharmacol Res Commun* **20**: 465–483, 1988.
31. Moroco JR, Solt DB and Polverini PJ, Sequential loss of suppressor genes for three specific functions during "*in vivo*" carcinogenesis. *Lab Invest* **63**: 298–306, 1990.