MOLECULAR PHARMACOLOGY, 24:60–68

Use of a Monoclonal Antibody for Comparative Studies of Monoamine Oxidase B in Mitochondrial Extracts of Human Brain and Peripheral Tissues

RICHARD M. DENNEY, RICHARD R. FRITZ, NUTAN T. PATEL, STEVEN G. WIDEN, AND CREED W. ABELL

Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77550

Received October 4, 1982; Accepted April 1, 1983

SUMMARY

Monoamine oxidase B (MAO B; EC 1.4.3.4) activity in detergent extracts of mitochondria from autopsy brain (gray matter and medulla), liver, lung, and kidney from a single individual and from pooled, human platelets could be immunoprecipitated by a monoclonal anti-human platelet MAO B antibody (MAO-1C2) in combination with appropriate secondary reagents. MAO A activity, which was detected in brain, liver, lung, and kidney, was not immunoprecipitated under the same conditions. All MAO B-containing extracts, regardless of tissue source, inhibited immunoprecipitation of [^{3}H]pargyline-labeled human platelet MAO, and the shapes of the inhibition curves were identical. The concentration of immunologically detectable MAO B protein in the extracts was estimated from immunoprecipitation competition data by reference to a standard curve relating observed inhibition of immunoprecipitation to the concentration of catalytically active platelet MAO added (estimated from [^{3}H]pargyline binding data). MAO B protein concentrations measured by this radioimmunoassay were similar to concentrations of active MAO B as measured by pargyline binding. These results demonstrate that in the brain and peripheral tissues studied, molecules with MAO B activity share a unique antigenic determinant and similar catalytic efficiency. They also extend previous observations that MAO B molecules extracted from mitochondria bear an antigenic determinant which is not present on MAO A molecules. These results demonstrate the validity of a new competitive radioimmunoassay for active plus inactive MAO B concentration in human platelet extracts and extracts of mitochondria from human tissues. This radioimmunoassay should complement [^{3}H]pargyline binding assays and enzyme activity assays in studies designed to clarify the mechanisms of genetic, disease, and treatment factors which lead to differences in MAO B function among individuals.

INTRODUCTION

MAO[1] (amine:oxygen oxidoreductase, EC 1.4.3.4), an integral component of the outer mitochondrial membrane (1, 2), is responsible for degrading a wide variety of biogenic amines to their corresponding aldehydes. The possible role of altered MAO activities in the etiology of certain psychiatric disorders has been debated for many years (reviewed in ref. 3). Numerous studies have reported reduced activities of platelet MAO in chronic schizophrenic patients as compared with normal subjects, but this reduction is not specific for schizophrenia. Abnormal platelet MAO activities have been reported in a variety of psychiatric and metabolic disorders and in nonpatients in association with psychiatric disturbance (3). To complicate the issue further, neuroleptic drug treatment recently has been shown to depress platelet MAO activities (3). Furthermore, the relationship of platelet MAO to MAO in the central nervous system has never been clearly described, as recently discussed by Fowler *et al.* (3).

A major unresolved question in both basic and clinical MAO research involves the structural and genetic rela-

This work was supported by United States Public Health Service Grant NIMH-34757, and by funds from the Cullen Foundation and the Multidisciplinary Research Program on Schizophrenia at the University of Texas Medical Branch in Galveston. We appreciate the cooperation of personnel of the Hybridoma Core Facility, operated by the University of Texas Medical Branch Cancer Center (National Cancer Institute Grant DHHS 5P30 CA-17701).

[1] The abbreviations used are: MAO, monoamine oxidase; 5-HT, 5-hydroxytryptamine; PEA, β-phenylethylamine; IgG, immunoglobulin G; SDS, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; RIA, radioimmunoassay; PBS, phosphate-buffered saline (containing, per liter, 0.2 g of KCl, 0.2 g of KH_2PO_4, 8 g of NaCl, and 1.144 g of Na_2HPO_4); RIA buffer (per liter), 0.05 M Tris buffer (pH 7.5), 0.14 M NaCl, 10^{-3} M EDTA, 0.05% NP-40, 0.1% bovine serum albumin, and 0.75% octylglucoside; logit function, ln[% unbound/(100 − % unbound)].

0026-895X/83/040060-09$02.00/0

tionship between the two types of MAO, A and B, which have distinct catalytic properties and are expressed in various proportions in different tissues, including brain (4–7). MAO A is selectively inhibited by low concentrations of the irreversible active-site inhibitor clorgyline and preferentially oxidizes low concentrations of 5-HT (4). MAO B is selectively inhibited by low concentrations of deprenyl (6) and preferentially oxidizes low concentrations of PEA (8) and benzylamine (4). The inhibitors clorgyline, deprenyl, and pargyline covalently bind to a single flavin moiety in one of the two subunits of the respective enzymes (9, 10). A third (and structurally unrelated) pyridoxal phosphate-containing enzyme, benzylamine oxidase, is highly specific for benzylamine and is not inhibited by either deprenyl or clorgyline (11).

Several laboratories have reported immunological comparisons of MAO A and MAO B and of MAO molecules derived from different tissues. McCauley and Racker (12) elicited an antiserum to bovine liver MAO (B enzyme) that specifically immunoprecipitated MAO B but not A activity from bovine brain. Similarly, Levitt *et al.* (13) have recently studied several antisera to bovine liver MAO B that appear to be specific for the B enzyme. In contrast, Dennick and Mayer (14) and Russell *et al.* (15) elicited antisera against purified human and rat liver MAO that did not discriminate MAO A from MAO B in the respective species. Craig *et al.* (16) and Brown *et al.* (17) studied the ability of antisera elicited in rabbits to human placental MAO (A) (18) or platelet MAO (B) (19) to cross-react with MAO from these and other human tissues. Antibodies in their antisera bound determinants on placental MAO A that were not present on platelet MAO B, but all antibodies which bound platelet MAO B also bound placental MAO A. Using techniques introduced by Köhler and Milstein (20), we recently isolated a hybridoma which secretes an antibody (MAO-1C2) with high specificity for human MAO B (21, 22). The hybridoma was isolated by fusion of a mouse myeloma cell line to spleen cells from a mouse immunized with human platelet MAO B. MAO-1C2 has the following interesting properties: (a) It binds both [^{3}H]pargyline-labeled, catalytically inactive human platelet MAO B and unlabeled, catalytically active MAO B from human platelets and liver. (b) Its binding to catalytically active human platelet MAO B has no marked effect on catalytic activity. (c) It precipitates human platelet and liver MAO B in the presence of appropriate secondary immunoglobulin reagents. (d) Immunoaffinity columns bearing MAO-1C2 appear to bind only MAO B from a complex mixture of proteins derived by detergent extraction of human liver mitochondria. MAO A activity from liver or placenta is not bound by these columns. (e) The antibody does not immunoprecipitate MAO A from human liver or placenta, or MAO A or B from rodent liver.

The studies reported here assess the ability of MAO-1C2 to bind MAO A and B molecules extracted from mitochondria of brain (gray matter and medulla), liver, lung, and kidney. The results demonstrate that MAO B molecules from these mitochondrial extracts share an antigenic determinant that is missing from MAO A in the same tissues, and have similar mean catalytic activities per molecule. The study also demonstrates the utility of MAO-1C2 in a competitive radioimmunoassay for MAO B concentration in extracts of platelets or of mitochondria from human tissues.

MATERIALS AND METHODS

Reagent sources. Rabbit anti-mouse IgG (heavy and light chain-specific) was purchased from Cappel Laboratories (Cochranville, Ill.). Heat-killed, formaldehyde-fixed *Staphylococcus aureus* cells and octyl-β-D-glucopyranoside (octylglucoside) were purchased from Calbiochem-Behring Corporation (La Jolla, Calif.). [^{3}H]Pargyline (specific activity 15 Ci/mmole), ^{14}C-labeled benzylamine (specific activity 14.6 mCi/mmole), PEA (specific activity 50 mCi/mmole), and 5-HT (specific activity 49.3 mCi/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.). Water-miscible scintillation fluid (PCS) was obtained from Amersham Radiochemical Corporation (Arlington Heights, Ill.). Other chemicals of reagent grade were obtained from standard sources.

Tissue sources. Pooled, outdated (36 to 72 hr old) human platelet-rich plasma was obtained from the blood bank of the University of Texas Medical Branch, Galveston, Tex. Human tissues were obtained from the autopsy service of Ben Taub Hospital (Houston, Tex.). All experiments involving brain, liver, lung, and kidney employed tissue obtained from a single 33-year-old male accident victim. The tissues were frozen within 16 hr of death and stored at −80° until thawed for isolation of mitochondrial pellets.

Preparation of extracts. Blood platelets from outdated platelet-rich plasma were prepared by the method of Corash (23) and extracted with 0.5% Triton X-100 (purified to remove oxidation products as described in ref. 24) in 0.05 M potassium phosphate buffer (pH 7.5). Mitochondria from human liver, lung, brain, and kidney were prepared by differential centrifugation as previously described for liver (22). MAO was extracted from mitochondria with 0.5% (v/v) Triton X-100 or 0.75% (w/v) octylglucoside in the same buffer used for platelets. Mitochondria, unlike platelets (see below), were not prewashed with 0.1% Triton X-100, to avoid low-level extraction of MAO activity in these samples.

Isolation and labeling of human platelet MAO. Platelet protein fractions of varying purity were derived from three different batches of outdated platelets (platelet batches 6, 7, and 8; Table 1). The properties of the relevant fractions are summarized in Table 1. The "crude soluble" fraction (platelet batch 8; see Table 1) was prepared by freezing and thawing a suspension of washed, pooled, outdated human platelets in 0.05 M potassium phosphate buffer (pH 7.5) and removing the insoluble material by centrifugation at 35,000 × g_{av} for 30 min at 4°. The "0.1% Triton X-100" fraction (Table 1) was prepared from the resulting pellet by extraction with 0.1% (v/v) purified Triton X-100 and clarification of the supernatant fluid by centrifugation as before. The "crude soluble" and "0.1% Triton X-100" fractions had very low MAO B activity (Table 1), and little or no immunologically detectable MAO protein by radioimmunoassay. In all three purification experiments, active MAO B was extracted with 0.5% (v/v) purified Triton X-100 from the residue which was insoluble in 0.1% Triton X-100. This 0.5% Triton X-100 extract, which was clarified by centrifugation as before, was denoted "crude MAO." MAO in the 0.5% Triton X-100 extracts from purification Experiments 7 and 8 was further purified by ammonium sulfate precipitation and DEAE-Sephacel chromatography (22) to yield fractions DEAE-7 and DEAE-8 (Table 1). Chromatofocused MAO was prepared by chromatographing DEAE-purified platelet MAO (from platelet batch 6) on a Polybuffer Exchanger 94 column (0.9 × 27 cm; Pharmacia Fine Chemicals, Piscataway, N.J.) according to instructions from the manufacturer. The pH gradient was developed by elution with 200 ml of eight times-diluted Polybuffer 74 (pH 4.0) containing 1% octylglucoside. The fractions with high MAO activity near pH 5.3 were pooled and precipitated by the addition of solid ammonium sulfate to 80% saturation. SDS-polyacrylamide gel profiles of the chromatofocused MAO preparation exhibited six prominent bands, including a major band (about 12% of the total staining by microdensitometry; see below) corresponding to a molecular weight of 59,000 (see ref. 22). The HPLC

TABLE 1

MAO activity and concentration in platelet protein fractions used

Fraction	Platelet batch	MAO			
		Total protein	Activity[a]	Specific activity	Specific concentration
		mg/ml	*units/ml*	*units/mg*	*μg/mg protein*
Triton X-100, 0.5%	6	5.5	510	92	ND[b]
Chromatofocused	6	0.24	130	520[c]	ND
HPLC	6	0.0065	26	3900[c]	ND
Triton X-100, 0.5%	7	9.2	750	82	ND
DEAE-7	7	5.8	6130	1060	8.4[d]
DEAE-7 ([^{3}H]pargyline)	7	1.7	530	310	8.4[d]
Crude soluble	8	2.8	23	8	<0.1
Triton X-100, 0.1%	8	2.2	65	29	<0.1
Triton X-100, 0.5%	8	5.0	592	120	ND
DEAE-8	8	6.0	9100	1500	16[d]
DEAE-8 ([^{3}H]pargyline)	8	1.8	820	450	16[d]

[a] Substrate, 2 mM benzylamine.

[b] ND, not determined.

[c] Purification of platelet MAO B by chromatofocusing and HPLC results in considerable loss of activity. Specific activities of these preparations as compared with cruder fractions underestimate the relative purification of total MAO B protein.

[d] Measured by [^{3}H]pargyline as described under Materials and Methods.

preparation was derived by chromatographing 1 ml (0.7 mg of protein) of chromatofocused platelet MAO B (platelet batch 6) on a Beckman Model 334 gradient liquid chromatograph using a SynChropak AX 300 column (4 × 300 mm), a Beckman Model 153 analytical UV detector containing a 280-nm filter, and an Altex C-RIA processor. The sample in 10 mM potassium phosphate buffer (pH 7.4) was injected into the column and eluted for 10 min with 100 mM potassium phosphate buffer (pH 7.4), for 10 min with a gradient of 0–1% (w/v) octylglucoside in the same buffer, and for 40 min with phosphate buffer containing 1% octylglucoside (flow rate of 1.0 ml/min). A peak of catalytically active MAO was located in the 1% octylglucoside fractions. The pooled fractions were concentrated by pressure ultrafiltration. SDS-polyacrylamide gel analysis and silver staining (see below) revealed two bands, one corresponding to the molecular weight of MAO (59,000), and the other to 28,000. The relative staining intensities of the two bands were about equal, as measured by microdensitometry (see below).

MAO activity assays. MAO was assayed for enzymatic activity by a radiometric assay modified from that of Wurtman and Axelrod (25). ^{14}C-Labeled benzylamine, ^{14}C-labeled PEA, and ^{14}C-labeled 5-HT were used at final substrate concentrations of 2 mM, 10 μM, and 100 μM, respectively. At these concentrations, 5-HT is primarily oxidized by MAO A, and PEA by MAO B. Reaction conditions were 30 min at 37°. Enzyme concentrations were adjusted so that reaction rates were linear over this time, and measured rates were proportional to enzyme concentrations. Benzylamine was isotopically diluted to a final specific activity of 2 mCi/mmole. PEA and 5-HT were used without isotopic dilution. Activities are expressed in units defined as nanomoles of substrate oxidized per hour. Specific activities are expressed as activity units per milligram of protein, as determined by the method of Lowry *et al.* (26).

Labeling of MAO with [^{3}H]pargyline. Labeling of DEAE-purified platelet MAO fractions with [^{3}H]pargyline (specific activity 15 Ci/mmole) was performed by incubating the enzyme fraction with 0.67 μM drug for 30 min at 37° and removing unbound label by dialysis against 0.01 M potassium phosphate buffer (pH 7.4) (22). Labeling of fractions DEAE-7 and DEAE-8 under these conditions resulted in approximately 70% reduction of MAO specific activity (Table 1). The nanomoles of labeled pargyline bound per milliliter of sample divided by the proportion of molecules of enzyme inactivated by the pargyline treatment provided an estimate of the concentration of catalytically active MAO B in the DEAE-purified preparations (Table 1). Specific concentration (micrograms of MAO per milligram of total protein) was calculated from [^{3}H]pargyline binding data, assuming one pargyline molecule bound per 120,000 molecular weight, using the equation,

$$\text{Specific concentration} = \frac{(\text{cpm/ml}) \times M_w}{P \times S \times E \times F_i \times (2.2 \times 10^9\ \text{dpm/mCi})}$$

where P = protein concentration in milligrams per milliliter, S = specific activity of pargyline in millicuries per micromole, E = counting efficiency (0.54 cpm/dpm in Beckman LS 8000), F_i = fraction of MAO B inactivated by pargyline labeling, and M_w = 120,000 μg/μmole for MAO B. Triton X-100 extracts of mitochondria from various tissues were labeled with [^{3}H]pargyline in the same way, except that the MAO A in these tissues was first inactivated by treatment with 0.5 μM clorgyline (30 min at 37°) and the labeled drug concentration was 0.9 μM. Under these conditions, the percentages of inactivation of the PEA-oxidizing activity in various extracts were 91% for liver, 70% for lung, 81% for gray matter, 96% for medulla, and 93% for kidney.

Antibody preparation. The isolation and characterization of mouse monoclonal anti-human MAO B (MAO-1C2) has been described (22). Antibody was prepared from ascites fluid by precipitation with ammonium sulfate (50% saturation) and exhaustive dialysis against PBS (containing, per liter, 0.2 g of KCl, 0.2 g of KH_2PO_4, 8 g of NaCl, and 1.144 g of Na_2HPO_4). The antibody preparation used here contained 20 mg of protein per milliliter and could bind the equivalent of 14 mg of MAO per milliliter.

Immunoprecipitation. Where indicated, antibody was diluted in PBS immunoprecipitation buffer (PBS plus 0.1% bovine serum albumin/0.75% octylglucoside), and incubations were carried out at 4° unless otherwise indicated. MAO B solubilized from mitochondria with Triton X-100 (0.4–4 units of PEA-oxidizing activity in 100 μl) was mixed with 25 μl of MAO-1C2 (1:50 dilution; 10 μg of protein) and incubated overnight. Rabbit anti-mouse IgG (20 μl containing 80 μg of specific antibody) was added for 1 hr, followed by 100 μl of heat-killed, fixed *Staphylococcus aureus* cells (27). After a further 15 min, tubes were centrifuged for 5 min in a Beckman Microfuge (approximately 10,000 × *g*). The supernatant fluid (245 μl minus the volume of *S. aureus* cells) was saved. The pellet was resuspended and washed three times with 1 ml of PBS immunoprecipitation buffer and finally resuspended in 245 μl of the same buffer. Samples of supernatant and resuspended pellet (immunoprecipitate) were assayed for MAO B activity, using 10 μM PEA as substrate.

RIA. All dilutions used RIA buffer adapted from that of Kessler (27) [0.05 M Tris buffer (pH 7.5), 0.14 M NaCl, 10^{-3} M EDTA, 0.05% NP-40,

and 0.1% bovine serum albumin] supplemented with 0.75% octylglucoside. Incubations were carried out in 96-well Cooke polystyrene microtiter plates at room temperature. Samples to be tested for competition were diluted to 100 μl with RIA buffer and mixed with [^{3}H]pargyline-labeled, DEAE-purified platelet MAO (DEAE 7; 25 μl of a 12-fold dilution, containing 3.6 μg of total protein and 30 ng of MAO, as measured by [^{3}H]pargyline binding capacity). A 1:2000 dilution of ammonium sulfate-precipitated MAO-1C2 (25 μl containing 0.25 μg of protein) was added, and the microtiter plates were sealed with tape and incubated overnight with gentle rotary shaking. Rabbit anti-mouse IgG (10 μl, containing 40 μg of specific antibody) was added, and the incubation was continued for 1 hr. Heat-killed, fixed *S. aureus* cells [50 μl of a 10% (w/v) suspension] were added, and after a further incubation for 15 min, the plates were centrifuged for 15 min at 1700 × g in an IEC centrifuge (Model PR-6) equipped with Dynatech microplate carriers (Alexandria, Va.). Samples of the clear supernatant (50–150 μl) were removed from each well, mixed with water-miscible scintillation fluid, and counted in a Packard Tri-Carb liquid scintillation spectrometer (counting efficiency 54%).

Calculation of RIA data. Specific concentrations of MAO (micrograms of MAO per milligram of protein) were calculated from competition data with an Apple II Plus microcomputer programmed for the purpose using Visicalc (Personal Software Inc., Sunnyvale, Calif.). The program expressed the competition data from the standard samples as ln[(% unbound)/(100 − % unbound)] (logit function), and calculated the least-squares parameters for the best straight line relating the logit-transformed data (dependent variable) to log (micrograms of MAO protein added) (Fig. 2) or log (units of MAO B activity added) (Fig. 3). The standard curve was roughly linear over the range of 25%–75% competition. The program then used the slope and intercept of the standard curve to calculate MAO B protein concentration in the unknowns by linear interpolation. DEAE-purified platelet MAO was used as standard because no pure MAO was available for this purpose. The concentration of MAO B protein in DEAE-purified standards was taken to be equal to the concentration of catalytically active MAO as measured by [^{3}H]pargyline binding.

SDS-polyacrylamide gel electrophoresis. SDS-polyacrylamide slab gels (7.5% running gel, 2.5% stacking gel, 0.5 mm thick) were prepared as described by Fairbanks *et al.* (28). Samples (50 μl) containing 40–1000 ng of protein were denatured by boiling for 5 min in 2% SDS/5% 2-mercaptoethanol. Proteins were stained with silver as described by Merrill *et al.* (29). Gels were scanned using a Joyce-Loebel scanning microdensitometer. Relative staining intensities of various bands were determined by measuring the areas under peaks of the scan.

RESULTS

Immunoprecipitation of MAO A and B activities in detergent extracts of mitochondria from various tissues. Triton X-100 extracts of mitochondria from brain (gray matter and medulla), liver, lung, and kidney were assayed for oxidation of 100 μM 5-HT and 10 μM PEA (Table 2). At these concentrations, 5-HT and PEA are selective, although not absolutely specific, for MAO A and B, respectively. Extracts of liver and kidney had the highest levels of PEA oxidation (225 and 205 nmoles/mg/hr, respectively), and the lung extract had the lowest activity (8 nmoles/mg/hr). PEA oxidation was greater than 5-HT oxidation in the brain extracts, whereas the reverse was true in liver, kidney, and especially lung. Control experiments verified that in extracts derived from all tissues except lung, more than 90% of the PEA-oxidizing activity was sensitive to 1 μM deprenyl and resistant to 1 μM clorgyline, whereas more than 90% of the 5-HT-oxidizing activity was sensitive to 1 μM clorgyline and resistant to 1 μM deprenyl (data not shown). The low PEA-oxidizing activity in the lung extract was 70% resistant to clorgyline and 75% sensitive to deprenyl, suggesting that some of the oxidation of PEA in this tissue was due to MAO A.

TABLE 2

MAO A and B activities in mitochondrial extracts from selected human tissues

Source of mitochondria[a]	Soluble protein	Substrate	
		Phenylethylamine[b]	5-HT[c]
	mg/ml	*nmoles/mg protein/hr*	
Brain			
Gray matter	1.6	34	18
Medulla	1.1	62	25
Liver	0.56	225	442
Lung	0.48	8	32
Kidney	0.58	205	276

[a] See Materials and Methods.
[b] Substrate concentration, 10 μM.
[c] Substrate concentration, 100 μM.

Samples of extracts of gray matter, medulla, liver, and kidney containing 3–4 units of PEA-oxidizing activity and of lung extract containing 0.44 unit of MAO B activity were subjected to indirect immunoprecipitation using MAO-1C2, and the precipitated and unprecipitated PEA-oxidizing activities were assayed (see Materials and Methods). The results in Table 3 show that, in all tissues except lung, more than 90% of the PEA-oxidizing activity was found in the immunoprecipitates, unless MAO-1C2 was omitted. Slightly less (70%) PEA-oxidizing activity was precipitated from the lung extract. Efficient precipitation of PEA-oxidizing activity required MAO-1C2, since only variable low levels of MAO B activity (2.6 to 12%) were detected in the pellets in experiments in which MAO-1C2 was omitted (Table 3). Similar experiments showed that the proportion of total 5-HT-oxidizing activity found in the pellet amounted to only 4% in gray matter, 5% in medulla, 8% in liver, 16% in lung, and 3% in kidney. These results are consistent with our previous reports on the specificity of the antibody for MAO B (21, 22).

Characterization of the quantitative RIA for MAO B protein. The ability of MAO-1C2 to immunoprecipitate MAO B activity from the tissues tested shows that MAO-1C2 binds MAO B from each extract. Competitive immunoprecipitation experiments were designed to compare the strength of binding of the antibody to platelet MAO B with that of its binding to MAO B in each extract.

Initial tests of the competitive assay employed platelet MAO preparations of varying purity. As expected, the addition of 1–80 μg of unlabeled, catalytically active, DEAE-purified platelet MAO (DEAE-7), which contained 8.4–670 ng of MAO protein (based on 8.4 μg of MAO protein per milligram of total protein as measured by [^{3}H]pargyline binding), resulted in a concentration-dependent inhibition of the immunoprecipitation of [^{3}H]pargyline-labeled MAO (Fig. 1). The minimal quantity of unlabeled MAO that gave measurable inhibition was approximately 17 ng (which corresponded to an MAO concentration in the extract of 170 ng/ml). In contrast, high concentrations of crude soluble platelet which lacked significant MAO activity failed to inhibit immu-

TABLE 3

Immunoprecipitation by MAO-1C2 of MAO B in mitochondrial extracts from selected human tissues

Portions of mitochondrial extracts were immunoprecipitated with MAO-1C2, and the resulting supernatants and pellets (immunoprecipitates) containing anti-mouse IgG and *Staphylococcus aureus* were assayed for PEA-oxidizing activity (10 μM substrate concentration) as described under Materials and Methods.

Source of MAO	Addition of MAO-1C2	Fraction assayed	MAO B activity[a]	% Precipitation[b]
			nmoles/hr/fraction	
Brain				
Gray matter	+	Supernatant	0.18 ± 0.01	
	+	Pellet	2.91 ± 0.27	94.2
	−	Supernatant	3.18 ± 0.42	
	−	Pellet	0.42 ± 0.02	11.7
Medulla	+	Supernatant	0.06 ± 0.00	
	+	Pellet	2.15 ± 0.27	97.4
	−	Supernatant	2.73 ± 0.10	
	−	Pellet	0.06 ± 0.01	2.2
Liver	+	Supernatant	0.21 ± 0.03	
	+	Pellet	2.74 ± 0.02	92.9
	−	Supernatant	3.71 ± 0.12	
	−	Pellet	0.10 ± 0.00	2.6
Lung	+	Supernatant	0.12 ± 0.01	
	+	Pellet	0.39 ± 0.16	76.5
	−	Supernatant	0.34 ± 0.20	
	−	Pellet	0.02 ± 0.00	5.8
Kidney	+	Supernatant	0.28 ± 0.07	
	+	Pellet	2.74 ± 0.16	90.8
	−	Supernatant	3.61 ± 0.08	
	−	Pellet	0.18 ± 0.18	4.8

[a] Values are reported as means ± standard deviation ($n = 3$).

[b] Calculated as activity in indicated fraction divided by total activity recovered in the pellet plus supernatant. Values were calculated by dividing the mean MAO B activity of the pellet by the sum of the mean activities in supernatant plus pellet.

noprecipitation of labeled MAO (Fig. 1). In addition, a 0.1% Triton X-100 extract of platelets, which had very low MAO activity (Table 1), also gave little or no inhibition (data not shown).

Logit-transformed data from similar tests of competition by the more highly purified MAO preparations are shown in Fig. 2. The logit-transformed competition curves of crude platelet extract and HPLC-purified MAO were similar in slope (least-squares slopes: 1.8 for crude platelet protein; 2.0 for the HPLC preparation), but the curve for the purified preparation was displaced 1.8 log units to the left of the corresponding crude platelet curve (Fig. 2A).

The similarity of the slopes suggests that the inhibition

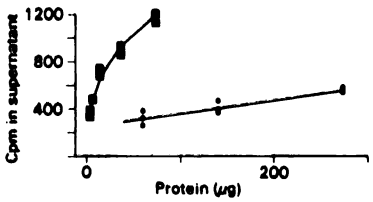

FIG. 1. *Inhibition of immunoprecipitation of [^{3}H]pargyline-labeled MAO by protein fractions from human platelets*

Varying amounts of protein from fractions derived from pooled, outdated human platelets (Table 1) were mixed with 3.3 μg of DEAE-purified human platelet MAO (DEAE-7; Table 1) containing 28 ng of MAO labeled to 70% saturation with [^{3}H]pargyline. Samples were immunoprecipitated with MAO-1C2, and the counts per minute remaining in the supernatant were determined as described under Materials and Methods. Competitors: ■—■, DEAE-7; •—•, crude soluble platelet fraction with very low MAO B activity (see Table 1).

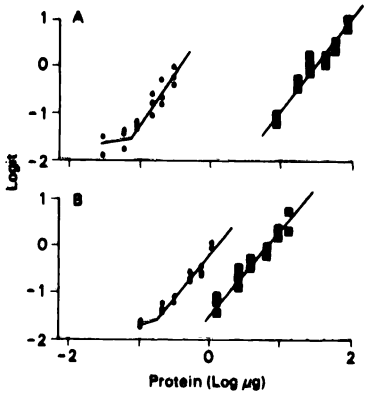

FIG. 2. *Inhibition of immunoprecipitation of [^{3}H]pargyline-labeled MAO by partially purified human platelet MAO*

Samples of competitors were mixed with [^{3}H]pargyline-labeled, DEAE-purified MAO (DEAE-7) and immunoprecipitated as described under Materials and Methods and in the legend to Fig. 1. Competition data was subjected to logit transformation as described under Materials and Methods. Source of competitors: A, HPLC-purified platelet MAO (Table 1), •—•; crude platelet extract, ■—■. B, chromatofocused platelet MAO (Table 1), •—•; DEAE-purified platelet MAO (DEAE-7, Table 1), ■—■.

of immunoprecipitation by the two fractions was due to the interaction of MAO-1C2 with a similar or identical determinant in each preparation. Since the relative position of the competition curves along the *X* axes in Fig. 3 (measured at logit = 0) was a measure of the relative competitive ability of each preparation per microgram of added protein, we derived estimates of the relative purity of the MAO-1C2 determinant in the preparations. These estimates were compared with estimates of the purity of MAO B protein in the same preparations as derived from comparisons of their specific activities. The results were as follows: (a) The 1.8-log unit difference in the positions of the competition curves from the HPLC-purified MAO preparation and the crude MAO extract (Fig. 3A) translates into a 68-fold higher MAO-1C2 determinant concentration in the HPLC preparation. This concentration difference should be compared with the 48-fold higher specific activity of the HPLC preparation (specific activity 82 for crude extract from platelet batch 7, 3900 for HPLC preparation from platelet batch 6; Table 1). (b) Similarly, the difference between the chromatofocused and DEAE-7 competition curves in Fig. 3B translates into an 8-fold higher MAO-1C2 determinant concentration in the chromatofocused preparation. This should be compared with the 6.3-fold higher specific activity of the chromatofocused preparation relative to the DEAE-7 fraction (Table 1).

Inhibition of immunoprecipitation of platelet MAO B by extracts from various tissues. Extracts of mitochondria from brain, liver, lung, and kidney were then compared with DEAE-purified platelet MAO (DEAE-7) for their ability to compete in the immunoprecipitation assay. All extracts showed inhibition, and the logit-transformed curves yielded similar slopes in the range of 25%–70% competition (Fig. 3). Note that, in Fig. 3, the logit-transformed competition data (*Y* axis) are plotted versus log (units of MAO B activity added), so that direct comparison of the positions of different curves along the *X* axis reflects the relative ability of a given amount of enzyme activity to inhibit immunoprecipitation.

Quantitation and measurement of the net molecular activity of MAO B using the competitive RIA. Since MAO-1C2 appeared to detect the same determinant on MAO B molecules in all tissues tested, quantitative comparisons of the inhibition of immunoprecipitation by each extract should constitute a valid RIA capable of measuring the concentration of the MAO B in the extracts. To test this competitive RIA, we compared MAO B protein concentrations measured by [^{3}H]pargyline binding assays with concentrations determined by the RIA, which used a standard curve relating the inhibition of immunoprecipitation to known concentrations of catalytically active MAO B in DEAE-purified platelet MAO (Fig. 4). The specific concentration of catalytically active MAO B protein in the standard DEAE-purified platelet MAO (8.4 μg of catalytically active MAO protein per milligram of total protein) was determined independently in [^{3}H]pargyline binding experiments. Estimates of MAO B concentration in extracts of medulla, liver, lung, and kidney agreed closely with the corresponding values derived from pargyline binding experiments (Table 4). The MAO B concentration estimated for gray matter by the competitive assay was approximately twice that estimated by pargyline binding assay, suggesting that there may have been more inactive MAO B protein in this extract.

By calculating the ratio of catalytic activity (expressed as molecules of substrate converted per milliliter per minute) divided by total (active plus inactive) enzyme concentration as determined by RIA (expressed as molecules of enzyme per milliliter), it was possible to measure the net molecular activity of the enzyme from various tissues. Table 4 also compares these net molecular activities with molecular activities of active enzyme, as calculated by the ratio of catalytic activity divided by active

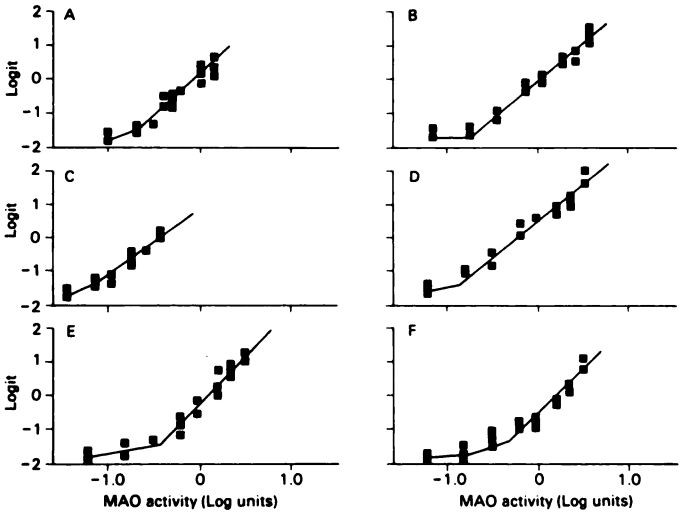

FIG. 3. *Inhibition of immunoprecipitation of [^{3}H]pargyline-labeled human platelet MAO by MAO B-containing extracts of mitochondria from various human tissues*

Samples of Triton X-100 extracts of mitochondria from human liver, lung, gray matter, medulla, and kidney or of DEAE-purified human platelet MAO were mixed with [^{3}H]pargyline-labeled, DEAE-purified MAO (DEAE-7) and immunoprecipitated as described in the legend to Fig. 1 and under Materials and Methods. Competition data were subjected to logit transformation (see Materials and Methods). The following is a list of competitors with the parameters of the best straight lines fitted to the data in the logit range from −1.0 to +1.0 by the method of least squares: A, liver (slope = 2.3 ± 0.1, intercept = 0.5 ± 0.05; B, lung (slope = 2.2 ± 0.2, intercept = 1.0 ± 0.1; C, gray matter (slope = 2.0 ± 0.1, intercept = 0.5 ± 0.05; D, medulla (slope = 2.8 ± 0.2, intercept = −0.2 ± 0.06; E, kidney (slope = 2.3 ± 0.22, intercept = −0.5 ± 0.07; F, DEAE-purified platelet MAO (DEAE-7, Table 1; slope = 2.1 ± 0.19, intercept = 0.0 ± 0.06).

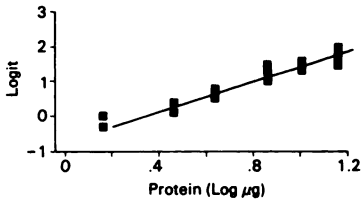

FIG. 4. *Standard curve for inhibition of immunoprecipitation of [^{3}H]pargyline-labeled MAO by DEAE-purified platelet MAO B of known concentration*

Varying amounts of DEAE-purified human platelet MAO (DEAE-7) were added to the immunoprecipitation mixture, and the immunoprecipitation of [^{3}H]pargyline-labeled MAO B was monitored by scintillation counting.

TABLE 4

Specific concentration and molecular activity of MAO B in selected human tissues as measured by pargyline binding and RIA

Tissue	Specific activity	Specific concentration		Molecular activity[a]	
		Pargyline binding[b]	RIA[c]	Pargyline binding[b]	RIA[c]
		μg MAO/mg		*moles PEA/min/mole enzyme*	
Brain					
Gray matter	34	1.4	2.8 ± 0.6	34	25 ± 5.2
Medulla	62	1.9	2.1 ± 0.5	40	63 ± 17
Liver	220	12	11 ± 1.3	36	42 ± 4.3
Lung	17	1.1	1.3 ± 0.0	30	26 ± 0.0
Kidney	210	9.2	12 ± 3.5	36	35 ± 8.5

[a] Substrate, 10 μM PEA.

[b] Calculated from the mean of duplicate assays of binding of [^{3}H]pargyline as described under Materials and Methods.

[c] DEAE-7 was used to generate a standard curve relating extent of competition to the quantity of DEAE-7 protein added. DEAE-7 had been independently found to contain 8.4 μg of active MAO B per milligram of total protein (see Table 1). Values are means of 12 determinations ± standard deviation.

site concentration as determined by titration with [^{3}H] pargyline. The net molecular activities in the different tissues varied from a low of 25 in gray matter to a high of 63 in medulla. Values for active enzyme molecular activity were similar, ranging from a low of 30 in lung to a high of 40 in medulla (Table 4).

DISCUSSION

We previously reported that MAO-1C2 immunoprecipitates MAO B from human platelets and liver, but not MAO A from human liver or placenta (22) and that immunoaffinity columns bearing covalently bound MAO-1C2 appear to bind most or all of the MAO B activity extracted from mitochondria in human platelets and liver (21). We have demonstrated here that MAO-1C2 can immunoprecipitate most MAO B molecules in extracts of mitochondria prepared from two regions of human brain (medulla and gray matter) and from two peripheral tissues (kidney and lung) in addition to platelets and liver (Table 2). Therefore, most or all MAO B molecules extracted from mitochondria in these tissues share a related antigenic determinant that is not present on MAO A molecules in the same tissues or in placenta. Immunoaffinity column experiments with mitochondrial extracts from human liver suggest that the MAO-1C2-defined determinant is found on all MAO B molecules detected in this tissue and is not present on other common detergent-extractable mitochondrial proteins from liver (21). Non-detergent extracts of platelet protein, which presumably lacked integral membrane proteins and had no significant MAO B activity, did not have significant levels of immunologically related protein. We did not systematically look for protein immunologically related to MAO in non-mitochondrial extracts from other tissues. It will clearly be of interest to use MAO-1C2 in a search for non-mitochondrial peptides that are immunologically and perhaps synthetically related to MAO.

The existence of an MAO B-specific determinant in humans is consistent with reports of MAO B-specific antisera to the bovine enzyme (12, 13). On the other hand, Craig *et al.* (16) and Brown *et al.* (17) concluded from studies of antisera to human placental MAO A and human platelet MAO B that there are determinants on the placental enzyme which are absent from the platelet enzyme. Since the placental enzyme has a slightly higher molecular weight (30), this observation raises the obvious possibility that MAO B is derived in some way from MAO A. The specificity of MAO-1C2 demonstrates that with human MAO, as with bovine MAO (12, 13), there is at least one determinant on MAO B which is not on MAO A.

The basic observation that MAO-1C2 can immunoprecipitate MAO B from mitochondria of various tissues suggests but does not prove that the shared determinants are identical. To examine the immunochemical similarity of the MAO-1C2-defined determinants on MAO B from different tissues, we tested the relative ability of MAO B from each tissue to compete with [^{3}H]pargyline-labeled human platelet MAO B for binding to the antibody. [Pargyline-labeled platelet MAO B was the original immunizing antigen (21, 22).] The competitive radioimmunoassay was based on our prior observation that MAO-1C2 binds equally well to catalytically inactive, [^{3}H]pargyline-labeled and catalytically active, unlabeled platelet MAO B (21, 22). Preliminary tests of the specificity of the competitive assay tested the ability of platelet MAO of varying degrees of purity to inhibit immunoprecipitation of [^{3}H]pargyline-labeled platelet MAO B. These tests demonstrated that the addition of large amounts of platelet protein fractions which were devoid of MAO had little or no inhibitory effect on immunoprecipitation by MAO-1C2 of [^{3}H]pargyline-labeled MAO B (Fig. 1). In contrast, addition of crude platelet extracts or preparations of partially purified platelet MAO B strongly inhibited the immunoprecipitation of [^{3}H]pargyline-labeled enzyme (Figs. 1 and 2).

The linearity of the logit-transformed competition data for platelet preparations of varying purity suggests that MAO-1C2 binds a single antigenic species in each preparation. Furthermore, the similarity of the slopes of the logit-transformed competition curves for all platelet-derived MAO preparations, regardless of their degree of purity (Fig. 2), suggests that the determinant recognized by the antibody was the same in each case. As expected, the extent of inhibition per microgram of added inhibitor protein increased with the purity of the competitor (Fig. 2).

Ideally, the relative effectiveness of a competitor

should be linearly related to the specific concentration of MAO in the various preparations. Since chromatofocusing and HPLC purification resulted in a somewhat greater increase in immunological purity (as measured by competitive ability per microgram of total protein) than in catalytic purity (as measured by specific activity), it appears that the antigenic activity of the determinant recognized by MAO-1C2 withstands chromatofocusing and HPLC better than does the catalytic activity of the enzyme molecule. Both purification procedures result in poor recoveries of catalytic activity.

Extracts of brain, liver, lung, and kidney also inhibited immunoprecipitation of [^{3}H]pargyline-labeled platelet MAO B. The slopes of the logit-transformed competition curves generated by all tissue extracts were similar to those of the platelet enzyme. We conclude that MAO-1C2 does not distinguish between MAO B from these tissues tested. The tight physical association of the MAO-1C2-defined determinant with MAO B activity and the apparent immunochemical identity of the determinant on MAO B from brain and peripheral tissues suggested that we could use platelet MAO B to calibrate a standard curve relating inhibition of immunoprecipitation to MAO B concentration. Unfortunately, no homogeneous preparation of the human platelet enzyme was available to serve as a standard. As an alternative, we used DEAE-purified platelet MAO B whose MAO B concentration was determined independently by titration with [^{3}H]pargyline of known specific activity. It should be noted that measurement of MAO concentration in this way measures only the catalytically active MAO. If the standard, whose MAO concentration was determined by [^{3}H]pargyline binding, contained some catalytically inactive but immunologically detectable MAO protein, we may have underestimated systematically the total immunologically detectable MAO protein by an amount equal to the proportion of catalytically inactive to total MAO B protein.

The RIA was then used to quantitate immunologically detectable MAO B protein in the mitochondrial extracts of the various tissues by reference to the platelet MAO standard curve. The data in Table 4 demonstrate that, in all tissues except gray matter, there was good agreement between values of MAO concentration as measured by [^{3}H]pargyline binding assays and immunologically detectable MAO concentration measured by the RIA. This suggests that the proportion of catalytically inactive to total MAO protein in most of the tissues was the same as that in the platelet preparation chosen as standard. The RIA appeared to detect about twice as much MAO protein in the gray matter extract as did the [^{3}H]pargyline binding assay, suggesting that the gray matter extract had higher levels of catalytically inactive but immunologically detectable MAO molecules than did the other extracts or the standard platelet MAO preparation. Whether this is an intrinsic property of MAO B extracted from mitochondria of gray matter or was due to greater degradation of the enzyme in this particular autopsy sample is not known.

Most previous estimates of the molecular activity or turnover number of MAO (molecules of substrate oxidized per minute per molecule of enzyme) have relied upon [^{3}H]pargyline, which detects only the catalytically active enzyme, to estimate the MAO B protein concentration (e.g., refs. 16 and 31). Because the RIA provided a way of estimating enzyme concentration independently of its catalytic activity, we were able to calculate the net molecular activity (molecules of substrate oxidized per minute per milligram of immunologically detectable MAO protein) of the enzyme from various human tissues. If there were regulatory mechanisms that result in large differences between tissues in the proportion of mitochondrial MAO B molecules which are catalytically active, we would have seen corresponding large differences in net molecular activity. Since the only differences in net molecular activity that we observed were small, the existence of such mechanisms appears to be unlikely.

The results presented here, which are based on a thorough study of the interaction of MAO-1C2 with MAO B in selected tissues from a single individual, suggest that MAO-1C2 may recognize human MAO B wherever it occurs. It is reasonable to ask to what extent these results can be generalized to the human population at large. Our published data which show that human liver MAO B binds MAO-1C2 (21, 22) were based on studies of autopsy liver tissue from three other individuals. In another series of experiments using tissues from a fourth individual, we found that MAO B activities in extracts of mitochondria from kidney and brain were also immunoprecipitable with MAO-1C2. We have also detected immunoprecipitable MAO B activity in diploid human skin fibroblasts (low levels), HeLa cells, and some clones of mouse-human hybrid cells generated by the fusion of a diploid human skin fibroblast with the mouse hepatoma cell line BWTG-3, described by Szpirer (32). Perhaps more conclusively, we have used the competitive RIA described here to quantitate MAO B protein concentration in platelet extracts from more than 100 individuals (including both psychiatric patients and normal subjects). The results show that the MAO-1C2-defined determinant is detectable in all samples tested.[2] From the data now available, we expect to detect the MAO-1C2-defined determinant in platelet extracts from most or all humans tested.

The RIA described here has a number of obvious basic and clinical applications in studies seeking to measure MAO B protein concentration independently of catalytic activity. For example, we have recently used the competitive RIA in experiments to determine that administration of a single dose of the potent MAO B inhibitor tranylcypromine (Parnate) to normal subjects results in an acute reduction of enzyme activity but no concomitant change in the steady-state concentration of extractable MAO B protein in platelets (33).

Population studies of MAO in various diseases have most frequently focused on the platelet enzyme because of its availability and because platelets have been suggested to be a model for central serotonergic neurons (reviewed in refs. 34 and 35). We are presently using both activity assays and the competitive RIA described here to measure the protein concentration and net molecular activity of MAO B in crude extracts of platelets from

[2] P. Malek-Ahmadi, J. A. Boeringa, S. Castellani, J. S. Uebersox, D. A. Lankford, R. M. Rose, R. R. Fritz, C. B. Denney, R. M. Denney, and C. W. Abell, in preparation.

normal subjects and drug-treated and drug-free psychiatric patients. These studies should provide important additional information about the mechanism by which genetic, disease, and treatment factors contribute to observed variations in platelet MAO B activity among individuals. Although numerous complex factors are involved in defining the relationship between platelet and brain MAO function (see ref. 3 for review), our data show that brain and platelet MAO B share an identical immunological determinant and a similar catalytic efficiency.

ACKNOWLEDGMENTS

We thank Dr. J. Knoll, Sammelweis University of Medicine, Budapest, Hungary, for his gift of deprenyl. We also thank Mrs. Vickie Kloeris and Miss Ann Waguespack for valuable technical assistance and Dr. Constance B. Denney for careful editing of the manuscript.

REFERENCES

1. Greenawalt, J. W., and C. Schnaitman. An appraisal of the use of monoamine oxidase as an enzyme marker for the outer mitochondrial membrane. *J. Cell Biol.* **46**:173–179 (1970).
2. Youdim, M. B. H. Biochemical characterization of the active site of brain monoamine oxidase. *Monogr. Neural Sci.* **7**:176–192 (1980).
3. Fowler, C. J., K. F. Tipton, A. V. P. MacKay, and M. B. H. Youdim. Human platelet monoamine oxidase—a useful enzyme in the study of psychiatric disorders? *Neuroscience* **7**:1577–1594 (1982).
4. Johnston, J. P. Some observations upon a new inhibitor of monoamine oxidase in human brain. *Biochem. Pharmacol.* **17**:1285–1297 (1968).
5. Squires, R. F. Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability: a comparison of eight species. *Adv. Biochem. Psychopharmacol.* **5**:355–370 (1972).
6. Knoll, J., and K. Magyar. Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv. Biochem. Psychopharmacol.* **5**:393–408 (1972).
7. Fowler, C. J., B. A. Callingham, T. J. Mantle, and K. F. Tipton. Monoamine oxidase A and B: a useful concept? *Biochem. Pharmacol.* **27**:97–101 (1978).
8. Yang, H.-Y., and N. H. Neff. *Beta*-phenylethylamine: a specific substrate for type B of monoamine oxidase of brain. *J. Pharmacol. Exp. Ther.* **187**:365–371 (1973).
9. Hellerman, L., and V. G. Erwin. Mitochondrial monoamine oxidase. II. Action of various inhibitors for the bovine kidney enzyme: catalytic mechanisms. *J. Biol. Chem.* **243**:5234–5243 (1968).
10. Singer, T. Active site-directed, irreversible inhibitors of monoamine oxidase, in *Monoamine Oxidase: Structure, Function and Altered Functions* (T. P. Singer, R. W. Von Korff, and D. L. Murphy, eds.). Academic Press, New York, 7–24 (1979).
11. Lewinsohn, R., K.-H. Bohm, V. Glover, and M. A. Sandler. A benzylamine oxidase distinct from monoamine oxidase B: widespread distribution in man and rat. *Biochem. Pharmacol.* **27**:1857–1863 (1978).
12. McCauley, R., and E. Racker. Separation of two monoamine oxidases from bovine liver. *Mol. Cell. Biochem.* **1**:73–81 (1973).
13. Levitt, P., J. E. Pintar, and X. O. Breakefield. Immunocytochemical demonstration of monoamine oxidase B in brain astrocytes and serotonergic neurons. *Proc. Natl. Acad. Sci. U. S. A.* **79**:6385–6389 (1982).
14. Dennick, R. G., and R. J. Mayer. Purification and immunochemical characterization of monoamine oxidase from rat and human liver. *Biochem. J.* **161**:167–174 (1977).
15. Russell, S. M., J. Davey, and R. J. Mayer. Immunochemical characterization of monoamine oxidase from human liver, placenta, platelets and brain cortex. *Biochem. J.* **181**:15–20 (1979).
16. Craig, I. W., J. F. Powell, G. K. Brown, and K. M. Summers. Studies on human monoamine oxidase, in *Monoamine Oxidase: Basic and Clinical Frontiers* (K. Kamijo, ed.). Excerpta Medica, Amsterdam, 18–27 (1982).
17. Brown, G. K., J. F. Powell, and I. W. Craig. Immunological studies of human monoamine oxidases. *J. Neurochem.* **39**:1266–1270 (1982).
18. Salach, J. I., and K. Detmer. Chemical characterization of monoamine oxidase A from human placenta mitochondria, in *Monoamine Oxidase: Structure, Function and Altered Functions* (T. P. Singer, R. W. Von Korff, and D. L. Murphy, eds.). Academic Press, New York, 121–128 (1979).
19. Donnelly, C. H., and D. L. Murphy. Substrate- and inhibitor-related characteristics of human platelet monoamine oxidase. *Biochem. Pharmacol.* **26**:853–858 (1977).
20. Köhler, G., and C. Milstein. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol.* **6**:511–519 (1976).
21. Denney, R. M., R. R. Fritz, N. T. Patel, and C. W. Abell. MAO A and B from human liver separated by immunoaffinity column chromatography using an MAO B-specific monoclonal antibody. *Science (Wash. D. C.)* **215**:1400–1403 (1982).
22. Denney, R. M., N. T. Patel, R. R. Fritz, and C. W. Abell. A monoclonal antibody elicited to human platelet monoamine oxidase: isolation and specificity for human monoamine oxidase B but not A. *Mol. Pharmacol.* **22**:500–508 (1982).
23. Corash, L. Platelet heterogeneity: relevance to the use of platelets to study psychiatric disorders. *Schizophr. Bull.* **6**:254–258 (1980).
24. Chang, H. W., and E. Bock. Pitfalls in the use of commercial nonionic detergents for the solubilization of integral membrane proteins: sulfhydryl oxidizing contaminants and their elimination. *Anal. Biochem.* **104**:112–117 (1980).
25. Wurtman, R. J., and J. Axelrod. A sensitive and specific assay for the estimation of monoamine oxidase. *Biochem. Pharmacol.* **12**:1439–1440 (1963).
26. Lowry, O. H., J. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
27. Kessler, S. W. Rapid isolation of antigens from cells with a staphylococcal protein A-antibody adsorbent: parameters of the interaction of antibody-antigen complexes with protein A. *J. Immunol.* **115**:1617–1624 (1975).
28. Fairbanks, G., T. L. Steck, and D. F. H. Wallach. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. *Biochemistry* **10**:2606–2617 (1971).
29. Merrill, C. R., D. Goldman, S. A. Sedman, and M. H. Ebert. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science (Wash. D. C.)* **211**:1437–1438 (1981).
30. Brown, G. K., J. F. Powell, and I. W. Craig. Molecular weight differences between human platelet and placental monoamine oxidase. *Biochem. Pharmacol.* **29**:2595–2603 (1980).
31. Giller, E., C. Castiglione, J. Wojciechowski, and X. O. Breakefield. Molecular properties of platelet MAO in psychiatric patients and controls, in *Biological Markers in Psychiatry and Neurology* (E. Usdin and I. Hanin, eds.). Pergamon Press, New York, 111–112 (1982).
32. Szpirer, C., and J. Szpirer. A mouse hepatoma cell line which secretes several serum proteins including albumin and alpha-foetoprotein. *Differentiation* **4**:85–91 (1975).
33. Fritz, R. R., P. Malek-Ahmadi, R. Rose, R. M. Denney, and C. W. Abell. Tranylcypromine affects platelet MAO B activity but not concentration. *Biol. Psychiatry* **18**:685–694 (1983).
34. Stahl, S. M. The human platelet. A diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. *Arch. Gen. Psychiatry* **34**:509–516 (1977).
35. Pletscher, A. Platelets as models for monoaminergic neurons. *Essays Neurochem. Neuropharmacol.* **3**:49–102 (1978).

Send reprint requests to: Dr. Richard M. Denney, Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Tex. 77550.