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SUBSTRATE SPECIFICITY AND INHIBITOR SENSITIVITY OF MONOAMINE OXIDASE IN RAT KIDNEY MITOCHONDRIA

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Abstract—The deamination of the substrates 5-hydroxytryptamine (5–HT), tyramine, dopamine, β-phenylethylamine and benzylamine by rat kidney mitochondrial monoamine oxidase (MAO) was studied, and kinetic constants are reported for each substrate. By the use of the selective MAO inhibitors, clorgyline and deprenyl, 5-HT and benzylamine were found to be substrates for types A and B MAO, respectively, in this tissue, whereas the other substrates were metabolized by both forms of MAO. No evidence for any significant metabolism of 5-HT or benzylamine by other amine oxidases was obtained. However, some conditions under which the carbonyl reagents semicarbazide, isoniazid and aminoguanidine may interfere with assays for MAO, without actually affecting enzyme activity directly, are described. Preincubation of kidney mitochondria with histamine resulted in a time- and oxygen-dependent irreversible inhibition of both type A and type B MAO activity; the exact nature of the inhibitory agent and its mode of action remain to be determined.

The properties of the outer mitochondrial membrane flavoprotein enzyme, monoamine oxidase (MAO), have been studied in a wide variety of animal tissues. The possibility has emerged from the results of previous investigations that MAO from a variety of sources may exist in multiple enzyme forms, which differ in characteristics such as substrate specificity, mobility upon polyacrylamide gels, and sensitivity to inhibitors and thermal denaturation, although at the present time the exact molecular basis for such differing properties remains unclear (see Refs. 1 and 2 for reviews).

Of particular recent interest for the study of multiple forms of MAO have been the observations that certain MAO inhibitors, such as clorgyline [3] and deprenyl [4], appear to distinguish between at least two forms of the enzyme, designated types A and B MAO, on the basis of their relative sensitivities toward these drugs. Original studies on MAO in rat brain and liver suggested that these enzyme forms might have well-defined substrate preferences in animal tissues with, for example, noradrenaline and 5-hydroxytryptamine (5-HT) being substrates for type A MAO, benzylamine and β-phenylethylamine being substrates for type B MAO, and some other substrates, such as tyramine, dopamine or tryptamine, possessing the ability to be metabolized by either enzyme form [5]. Although these substrate specificities have been confirmed in several other animal tissues, a number of exceptions have arisen of tissues in which, for instance, benzylamine and β-phenylethylamine are substrates for type A, and 5-HT is a substrate for the type B enzyme (see Ref 6. for review). Consequently, it is clear that the properties of MAO types, with regard to these factors, are not necessarily the same from one animal source to the next. For this reason, the characterization of MAO in tissues which have undergone little or no previous investigation is essential in order to provide a better perspective of the similarities and differences between monoamine oxidases from different sources.

The metabolism of amines by MAO has been studied previously in many rat organs by various workers. However, the kidney is one rat tissue for which detailed information about this subject is lacking. The present paper, therefore, represents a more extensive study of amine metabolism *in vitro* by MAO in rat kidney mitochondria, with particular regard to the possibility of multiple forms of the enzyme, detectable by selective MAO inhibitors, being present within this tissue. In addition, the possibility has also been considered in this work that the deamination of monoamine substrates by kidney mitochondria may be brought about by amine oxidases distinct from the flavin containing enzyme.

MATERIALS AND METHODS

The radioactive substrates used were [G-^{3}H]-5-hydroxytryptamine creatinine sulfate and [ring-G-^{3}H] dopamine hydrochloride from Amersham-Searle, Arlington Heights, IL; [G-^{3}H] tyramine hydrochloride and β-[ethyl-1-^{14}C]phenylethylamine hydrochloride from New England Nuclear, Boston, MA; and [methylene-^{14}C]benzylamine hydrochloride from ICN Pharmaceuticals Inc., Irvine, CA. Corresponding unlabeled forms of these amine salts were obtained from the Sigma Chemical Co., St. Louis, MO, except for benzylamine hydrochloride which was purchased from K & K Labs, Plainview, NY.

Clorgyline [*N*-methyl-*N*-propargyl-3-(2, 4-dichlorophenoxy) propylamine] hydrochloride was kindly provided by May & Baker Ltd., Dagenham, Essex, England, and (−)-deprenyl [phenylisopropylmethyl propinylamine] hydrochloride was a gift from Dr. P. H. Kelly, Department of Pharmacology, Michigan State University, East Lansing, MI.

Other compounds (aminoguanidine hemisulfate, semicarbazide hydrochloride, isoniazid and histamine dihydrochloride) were purchased from the Sigma Chemical Co.

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Preparation of mitochondria. Rat kidney mitochondria were prepared routinely from pooled tissues of four to six male Charles River rats weighing around 250 g. The rats were killed by cervical dislocation, and the kidneys were removed, blotted and weighed. The thin connective tissue capsule was stripped from around each kidney and the visible remaining portions of the major blood vessels and the ureter leaving the organ were dissected away. The pooled tissue was minced with scissors and washed with ice-cold buffer (0.25 M sucrose, 0.001 M potassium phosphate, pH 7.8), and then homogenized in 10 vol. of this same buffer, using a conical ground glass hand homogenizer. The homogenate was centrifuged at 600 *g* for 10 min in a Sorvall RC-2 refrigerated centrifuge, in order to remove cell debris. The supernatant faction was carefully decanted and then centrifuged at 15,000 *g* for 20 min. The resulting mitochondrial pellet was resuspended in isolation buffer to give a final protein concentration of 13.5 mg/ml, measured by the biuret method of Gornall *et al.* [7], using bovine serum albumin as a standard. The mitochondrial preparation was divided into individual aliquots of 2–3 ml, which were stored at −20° for subsequent use in experiments within 2–3 weeks. MAO activity remained stable over this period. The results described in this paper were obtained by the use of several different preparations of mitochondria, isolated as described above during the course of these studies. By preparing and storing different batches of mitochondria in this identical manner, MAO activities and experimental results were found to be reproducible from one preparation to the next.

Assay of MAO. The assay of MAO activity was based on the radiochemical method of Callingham and Laverty [8]. The radiochemical substrates were used at final specific activities of 0.5 μCi/μmole (benzylamine and β-phenylethylamine), 1.0 μCi/μmole (tyramine and dopamine) and 2.0 μCi/μmole (5-HT), obtained by diluting the radiolabeled compounds with aqueous solutions of their corresponding non-labeled amine salt, to give stock solutions of 100 mM which were stored at −20°. For use in assays, appropriate aliquots of the stock solutions were diluted with 0.2 M potassium phosphate buffer, pH 7.8, in order to give radioactive substrate solutions which yielded the desired final amine concentration when included in the assay.

Briefly, ice-cooled assay tubes contained 25 μl of mitochondrial preparation, 25 μl of distilled water and 50 μl of radioactive substrate solution. When solutions of inhibitor drugs were also used in the assay, the volume of distilled water was reduced correspondingly in order to retain a total assay volume of 100 μl. All tubes were flushed with oxygen, capped with rubber stoppers and incubated for 10 min at 37°, before stopping the reaction by cooling rapidly in ice and by the addition of 10 μl of 3 N HCl to each tube. For blank assays, the addition of HCl to the assay mixture preceded the incubation period. Deaminated metabolites in each tube were extracted into 0.5 ml ethyl acetate–benzene (1:1, v/v), and 0.4 ml of the organic phase was counted for radioactivity in a Beckman LS-230 liquid scintillation spectrometer using 12 ml of 0.4% butyl-PBD [2-(4′-tert-butylphenyl)-5-(4″-biphenylyl)-1, 3, 4-oxadiazole] in toluene (w/v) as scintillant. All counts were corrected for quenching and converted to dis./min for calculation of results. The production of metabolites from each amine substrate was found to remain linear as long as the mitochondrial aliquot and incubation time used in the assay did not exceed those values described above.

Studies with inhibitors. For inhibition studies involving clorgyline and deprenyl, 10 μl of appropriate aqueous solutions of these drugs were added to the mitochondria and distilled water to give a total preincubation volume of 50 μl. These samples were preincubated for 20 min at 37°, and then rapidly ice-cooled before the addition of 50 μl of radioactive substrate for assay of MAO as described above.

The effects of the other compounds used (aminoguanidine, isoniazid, semicarbazide and histamine) were studied in the same way, except that drug solutions were prepared in 0.2 M potassium phosphate buffer, pH 7.8 (appropriate volumes of this buffer were also included in corresponding control assay preincubation mixtures containing no inhibitors), and the preincubation periods were varied in some experiments as described within the text.

All inhibitor concentrations quoted in this paper represent, when appropriate, the concentrations of the drugs present during the preincubation period.

RESULTS

Kinetic constants for rat kidney mitochondrial MAO. The apparent K_m and V_{max} values for MAO activity toward each substrate were determined by measuring initial MAO reaction velocities for rat kidney mitochondria at different substrate concentrations, and by analysis of the resulting data by the double-reciprocal Lineweaver–Burk method. All Lineweaver–Burk plots were linear over the range of substrate concentrations studied (5 μM to 2.5 mM). The kinetic constants obtained for each substrate are shown in Table 1. Subsequent experiments described in this paper were performed at final substrate concentrations of 1 mM, which represents approximately 6–20 times the estimated K_m concentrations, depending upon the substrate used.

Inhibition of rat kidney mitochondrial MAO by clorgyline. The sensitivity of rat kidney MAO toward inhibition by clorgyline was investigated using each of the five substrates: 5-HT, tyramine, dopamine, β-phenylethylamine and benzylamine. Mitochondria were preincubated with varying concentrations of clorgyline for 20 min at 37° before addition of substrate (1 mM

Table 1. Kinetic constants for rat kidney mitochondrial MAO

Substrate	K_m* (μM)	V_{max} (nmoles/hr/mg protein)
Benzylamine (7)†	98 ± 5	17 ± 2
5-HT (7)	109 ± 7	40 ± 6
Tyramine (7)	141 ± 5	54 ± 5
Phenylethylamine (3)	46 ± 3	64 ± 10
Dopamine (3)	166 ± 17	55 ± 11

* Apparent Michaelis–Menten constants (K_m) and maximal velocities (V_{max}) are reported as the means ± S.E. of values from all experiments.

† Figures in parentheses represent the numbers of different experiments, each yielding a single estimation of the kinetic constants.

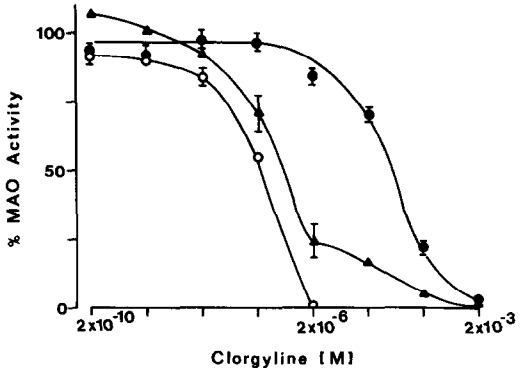

Fig. 1. Inhibition of rat kidney mitochondrial MAO by different clorgyline concentrations. Remaining MAO activity was assayed at final substrate concentrations of 1×10^{-3} M after a 20-min preincubation at 37° with clorgyline. Key: O——O, 5-HT, ▲——▲, tyramine; and ●——●, benzylamine as substrate. All points represent the mean of triplicate determinations (standard errors are shown when they exceed symbol size).

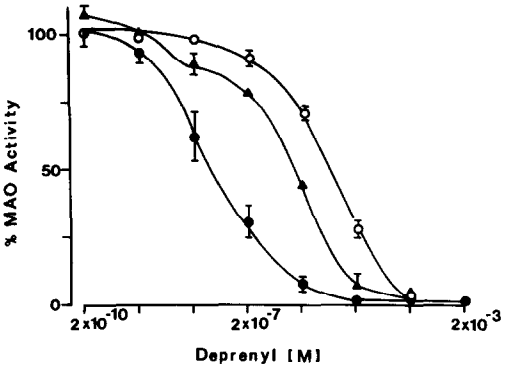

Fig. 3.Inhibition of rat kidney mitochondrial MAO by different deprenyl concentrations. Remaining MAO activity was assayed at final substrate concentrations of 1×10^{-3} M after a 20-min preincubation at 37° with deprenyl. Key: O——O, 5-HT; ▲——▲, tyramine; and ●——●, benzylamine. All points represent the mean of triplicate determinations (standard errors are shown when they exceed symbol size).

final assay concentration) to measure remaining MAO activity. Enzyme activities toward each substrate were expressed as percentages of uninhibited control values, and are plotted in Fig. 1 and 2 against clorgyline concentrations (on a logarithmic scale).

The inhibition curve for MAO activity toward 5-HT was single-sigmoidal, with complete inhibition being reached at a clorgyline concentration of 2×10^{-6} M (Fig. 1). Benzylamine metabolism was also inhibited in a single-sigmoidal fashion, but in this case, higher concentrations of clorgyline were required, with complete inhibition only occurring at 2×10^{-3} M (Fig. 1). On the basis of the original studies of Johnston [3] defining types A and B MAO as a result of their differing relative sensitivities toward clorgyline, the present results indicate that 5-HT is a substrate for type A MAO and benzylamine is a substrate for type B MAO in rat kidney mitochondria.

In contrast, the inhibition curves for tyramine (Fig. 1), dopamine and β-phenylethylamine (Fig. 2) metabolism were biphasic, spanning the whole range of clorgyline concentrations used, and also containing a plateau region at intermediate concentrations. Thus, each of these substrates can be metabolized by both type A and type B MAO in rat kidney mitochondria. Under the *in vitro* conditions studied, approximately 60 per cent of β-phenylethylamine metabolism and 80 per cent of tyramine and dopamine metabolism were brought about by the type A enzyme component in this tissue.

Inhibition of rat kidney mitochondrial MAO by deprenyl. Studies similar to those using clorgyline were also performed with another MAO inhibitor, deprenyl. Figures 3 and 4 show the inhibition curves obtained when each of the five different substrates (at 1 mM) was used to assay MAO activity in the presence of varying concentrations of the inhibitor.

As with clorgyline, both 5-HT and benzylamine metabolism were inhibited in a single-sigmoidal fashion by deprenyl (Fig. 3). However, with this latter drug, MAO activity toward benzylamine was the more sensitive to inhibition. Inhibition of 5-HT metabolism required approximately a 100-fold higher deprenyl concentration than did inhibition of benzylamine metabolism. Using the original observations of Knoll and Magyar [4] describing the relative apparent sensitivities of types A and B MAO to inhibition by de-

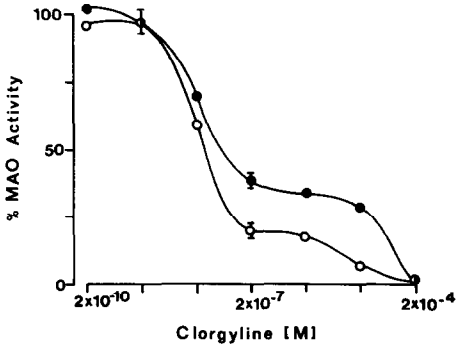

Fig. 2. Legend as in Fig. 1 except for the key: O——O, dopamine; and ●——● β-phenylethylamine.

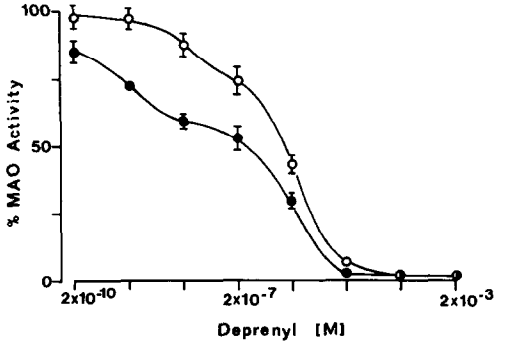

Fig. 4. Legend as in Fig. 3 except for the key: O——O, dopamine; and ●——●, β-phenylethylamine.

prenyl, the present results confirm the conclusions from the previous section, that 5-HT is a substrate for type A and benzylamine is a substrate for type B MAO in rat kidney mitochondria.

Furthermore, the corresponding inhibition curves obtained with the substrates tyramine, dopamine and β-phenylethylamine were again double-sigmoidal (Figs. 3 and 4). The position of the plateau regions indicates that type A MAO was responsible for approximately 85, 80 and 55 per cent respectively, of the total metabolism of these substrates under these conditions. These results thus agree well with similar conclusions obtained from the studies using clorgyline.

Effects of carbonyl reagents and histamine upon amine metabolism in rat kidney mitochondria. The oxidative deamination of some monoamines can be brought about not only by mitochondrial MAO, which is a flavoprotein enzyme, but also by a group of amine oxidases (diamine oxidase, plasma and connective tissue amine oxidase) which are believed to contain copper and possibly pyridoxal phosphate as their co-factors [9]. The enzymes of this latter group generally appear to exist in a soluble form, and have been reported to be distinguishable from mitochondrial MAO by their sensitivities to inhibition by carbonyl reagents such as semicarbazide, isoniazid and aminoguanidine [9–11], and by their resistance to inhibition by acetylenic MAO inhibitors such as clorgyline at concentrations which are able to inactivate completely the flavin-containing MAO activities [12, 13]. The studies on amine metabolism described so far in this paper involved the use of mitochondrial preparations, and, in addition, the data showing complete inhibition of amine metabolism by clorgyline and deprenyl at concentrations of $2 < 10^{-3}$ M gave no suggestion that deamination of any substrate used was brought about by an enzymic system other than mitochondrial MAO. However, it was considered important, nonetheless, to determine whether several carbonyl reagents could affect the metabolism of some amines in rat kidney mitochondria. The substrates 5-HT and benzylamine were used in these studies. In addition, since the kidneys of several animal species had been shown previously to possess histaminase or diamine oxidase (DAO) activity [14], possible effects of histamine upon the metabolism of 5-HT and benzylamine were also investigated in these experiments.

Preliminary studies, in which the carbonyl reagents or histamine were preincubated at concentrations of 2×10^{-3} M for 20 min at 37° with mitochondria, indicated that these compounds could, indeed, cause an apparent inhibition of both 5-HT and benzylamine deamination, depending upon the reagent employed. Before reaching the conclusion that these results indicated a direct effect of these compounds upon amine metabolism, we considered two other possibilities that might have given rise to an "artifactual" inhibition of the activities measured.

The first possibility, that inclusion of these compounds in the assay might alter the pH of the assay medium, could be discounted as long as they were prepared in buffer solutions at the assay pH 7.8 (see Materials and Methods). However, changes in pH did become a problem in some cases if non-buffered solutions of the compounds were used instead.

A second possibility was that the carbonyl reagents might form adducts with the aldehyde product resulting from deamination of amine substrates by mitochondrial MAO and thus affect the extraction of metabolites by the organic solvent mixture used in the assay. This was tested experimentally by comparing apparent enzyme activities from assays in which the compounds were present throughout the preincubation and subsequent assay procedure, with those activities measured when

Table 2. Effects of various drugs on the assay of MAO activity in rat kidney mitochondria*

Drug	MAO Activity (%) (5-HT)		MAO Activity (%) (Benzylamine)	
	10 min	30 min	10 min	30 min
Semicarbazide (+)	86 ± 3	109 ± 2	99 ± 2	96 ± 1
Semicarbazide (−)	78 ± 4	[illegible]	94 ± 5	95 ± 1
Isoniazid (+)	85 ± 3	128 ± 3	82 ± 1	93 ± 3
Isoniazid (−)	78 ± 4	78 ± 2	79 ± 4	81 ± 1
Aminoguanidine (+)	8 ± 1	22 ± 1	13 ± 3	26 ± 1
Aminoguanidine (−)	43 ± 2	48 ± 1	80 ± 2	83 ± 2
Histamine (+)	73 ± 3	73 ± 1	83 ± 2	78 ± 1
Histamine (−)	99 ± 2	103 ± 4	97 ± 3	97 ± 1

* All samples were preincubated for 20 min at 37°, some (+) containing the appropriate drugs at a preincubation concentration of 2×10^{-3} M, and then incubated for 10 or 30 min after the addition of the appropriate substrate (1×10^{-3} M final concentration) to assay MAO activity. Other samples (−) contained no drugs during the preincubation period or the assay reaction, but instead corresponding amounts of the drugs (10 μl of appropriate drug solutions) were added after termination of the reaction with HCl. All values are reported as percentages of activities of corresponding control samples which contained all other constituents except for the drugs during these procedures, and represent the means ± S.E. of triplicate determinations on each sample. Control activities (dis./min of extracted metabolites) were: 20,117 ± 710 (10 min) and 30,008 ± 375 (30 min) for 5-HT; 1,197 ± 28 (10 min) and 3,209 ± 86 (30 min) for benzylamine.

corresponding amounts of these compounds were instead added after the termination by HCl of control assay reactions which contained none of the inhibitors. Otherwise these control samples had been subjected to an identical preincubation and assay schedule. By this experimental design we hoped to evaluate whether or not the apparent effects of these various compounds required their presence during the actual enzyme reaction. Results are shown for both 5-HT and benzylamine deamination, measured after assay reaction times of 10 and 30 min (Table 2).

From the data obtained with an assay reaction time of 10 min, all of the compounds, when present throughout the assay procedure, gave rise to varying degrees of inhibition of enzyme activity toward the two substrates, except in the case of semicarbazide which appeared to have no significant effect on benzylamine metabolism. However, the inhibitory effects of semicarbazide and isoniazid were also reproduced in those samples to which the compounds were added after the termination of the enzymic reaction. Thus, these agents probably are affecting the extraction of reaction products in the assay, rather than directly inhibiting amine metabolism. On the other hand, although aminoguanidine also may have acted to some extent on metabolite extraction, there was consistently greater enzyme inhibition when the inhibitor was present during the whole assay, suggesting that this compound may be acting directly upon amine metabolism as well. The effects of histamine in this experiment were more easily distinguishable. Addition of histamine after termination of the assay reaction did not affect enzyme activity, whereas its presence during the reaction resulted in a significant inhibition of activity toward both 5-HT and benzylamine.

The experimental results obtained when the deamination reaction was allowed to proceed for 30 min were qualitatively similar in many respects with those obtained after 10 min, except for the surprising finding that 5-HT deamination appeared to be greater in the presence of semicarbazide and isoniazid than in control samples. This observation was re-examined in greater detail by following the time course of 5-HT metabolism in the presence and absence of both drugs (Fig. 5).

In control samples, metabolite production was linear for 10 min and then deviated sharply from linearity at longer times. Metabolite production was also linear, although at a slower rate, in the presence of semicarbazide and isoniazid during this initial 10-min period. Addition of these compounds to "terminated" control enzyme assays indicated that the apparent inhibition of metabolite production during the initial 10-min period, seen in samples containing these drugs throughout the assay, could be explained entirely by the ability of these agents to reduce the extraction of the products formed (data not shown). However, this reduced apparent reaction rate remained linear for a longer period than did the control rate, and consequently, after incubation time of 15 min and longer, enzyme activity appeared to be greater than controls when these compounds were present during the enzymic reaction. Some possible reasons for these observations are suggested later (see Discussion).

Further characterization of the inhibition caused by histamine. Figure 6 shows the inhibition of 5-HT and benzylamine deamination resulting from preincubation of histamine with mitochondria for varying periods. Inhibition by histamine (2×10^{-3} M) was time-dependent and resulted in a progressively greater inhibition of 5-HT, rather than benzylamine, metabolism with increasing preincubation time. In the absence of preincubation, no significant inhibition was observed. Lower concentrations of histamine (2×10^{-5} and 2×10^{-4} M) in this experiment had much smaller inhibitory effects after the single 90-min preincubation time studied. In order to observe these inhibitory effects of histamine, a possible requirement for aerobic conditions during preincubation was investigated in a separate experiment. After a 60-min preincubation of histamine ($2 < 10^{-3}$ M) with mitochondria at 37° in air, 5-HT and benzylamine deamination were inhibited by 57 ± 1

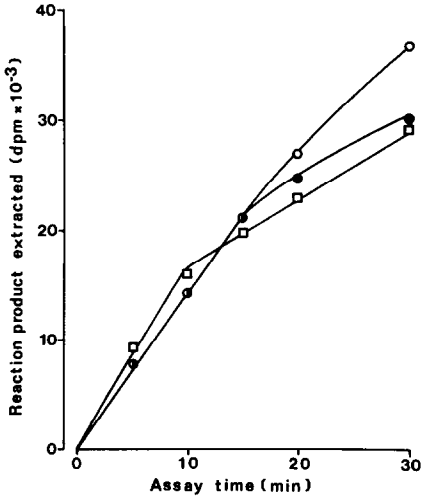

Fig. 5. Total reaction products extracted from assays for MAO activity toward 5-HT, using rat kidney mitochondria which had been preincubated for 20 min at 37° in the presence or absence of carbonyl reagents (2×10^{-3} M). Key: □——□, controls; ●——●, + semicarbazide; ○——○, isoniazid. Each point is the mean of duplicate determinations.

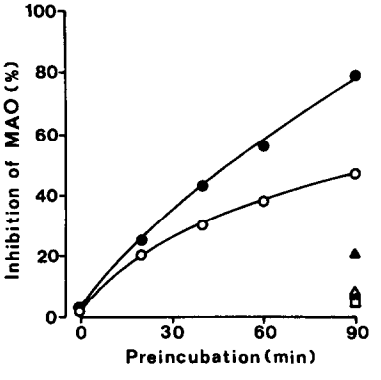

Fig. 6. Influence of preincubation time upon inhibition of rat kidney MAO by histamine. Key: preincubation concentrations of histamine were: 2×10^{-3} M (● and ○); 2×10^{-4} M (▲ and △); and 2×10^{-5} M (■ and □). Solid symbols denote 5-HT, and open symbols benzylamine used to assay remaining MAO activity. Each point is the mean of triplicate determinations (no standard errors > ± 7 per cent).

per cent and 44 ± 2 per cent (mean ± S. E. of triplicate determinations), respectively, compared with their corresponding controls. On the other hand, when identical preincubation mixtures were pre-gassed by bubbling N_2 through them, and then preincubated in closed, N_2-filled vessels at the same time as the aerobic samples described above, the inhibitory action of histamine upon metabolism of both amines was abolished completely. Thus, it is clear that oxygen is necessary for this action of histamine. Finally, we determined whether the amine metabolism in the mitochondrial fractions was inhibited reversibly or irreversibly by preincubation with histamine. In these experiments, mitochondria were preincubated for 60 min at 37° in the presence and absence of histamine ($2 < 10^{-3}$ M). Then some control and inhibited samples were subjected to an extensive dilution and washing procedure involving two re-isolation steps to recover the mitochondria by centrifugation, as described in the legend to Table 3. The deamination of 5-HT and benzylamine in these samples was compared with similar samples which had undergone the preincubation schedule, but not the subsequent washing and re-isolation procedure. The data from two separate experiments (Table 3) indicated that, after preincubation with histamine, the inhibition of 5-HT metabolism in washed samples was virtually identical to that in unwashed samples, indicating that the dilution, washing and re-isolation of the mitochondria failed to reverse the observed inhibition. With benzylamine, a partial reversal of the inhibition of its metabolism was obtained after washing in each experiment, but the major part of the inhibition still appeared to be largely irreversible.

Table 3. Effects of dilution, washing and reisolation of mitochondria upon the inhibition of rat kidney MAO by histamine*

Substrate	Type of sample	MAO activity (% of uninhibited controls) Expt. 1	Expt. 2
5-HT	Unwashed	42 ± 2	38 ± 1
	Washed	44 ± 1	41 ± 1
Benzylamine	Unwashed	57 ± 1	58 ± 2
	Washed	67 ± 3	67 ± 1

* Reaction mixtures consisted of 1 ml mitochondrial preparation and 1 ml buffer (0.2 M potassium phosphate, pH 7.8) containing histamine (final concentration 2×10^{-3} M). All mixtures were preincubated for 60 min at 37° and then rapidly ice-cooled. Some samples were diluted to a total volume of 20 ml with ice-cold buffer and then centrifuged at 30,000 *g* for 45 min (at 0°). The mitochondrial pellets were resuspended in 20 ml of ice-cold buffer, recentrifuged at 30,000 *g* for 1 hr, and the resulting pellets were each resuspended in 1 ml buffer. The remaining MAO activity was measured at 1×10^{-3} M substrate concentrations for samples obtained with and without the dilution, washing and re-isolation procedure. In each case, MAO activity is expressed as a percentage of the appropriate control activities which contained no histamine. Each value is the mean ± S.E. of triplicate determinations on each sample type. Results from two separate experiments are shown.

DISCUSSION

The experiments described in this paper show that rat kidney mitochondrial MAO can deaminate a variety of monoamines *in vitro*, and that this organ contains both type A and type B MAO activities described previously in other tissues on the basis of differential sensitivities toward the inhibitors, clorgyline and deprenyl (e.g. Refs. 3–6). In particular, the present work confirms and extends the original observations of Squires [15], based on selective inhibition of kynuramine metabolism, that both MAO types are present in this tissue. In most respects, the substrate specificities found here for the two MAO forms in rat kidney are identical to results obtained previously with rat liver and brain [3–5]. However, an exception was found with our data for β-phenylethylamine. Although, on the basis of original studies with rat brain, this amine has been assumed, for some years, to be a substrate solely for type B MAO [5], our results show that it can also be metabolized by the type A enzyme in rat kidney. This substrate is also metabolized by type A MAO of rat heart [13], and additional examples of similar findings in tissues from other species have been reviewed recently [6]. Thus, the present studies further underline the recent conclusions that previously accepted rigid classifications of the substrate specificities of types A and B MAO in animal tissues are not applicable universally [6].

There is now good evidence that the different MAO types may represent binding of different types or amounts of phospholipid material to a single enzyme protein in the outer mitochondrial membrane [16, 17]. Since it is clear that the respective substrate specificities of types A and B MAO are not identical when studied from different animal sources [6], it seems probable that the nature of the membrane micro-environment surrounding each of these enzyme forms may vary from tissue to tissue, perhaps resulting in some degree of heterogeneity within these two types of enzymic activities. Indeed, evidence for multiple forms of type B MAO within a single tissue, the pig heart, has been presented recently [18]. Varying properties of MAO activity have also been described previously within mitochondrial populations [19–21] and within different types of cells [22, 23]; these latter findings might be of relevance to the kidney, a tissue which contains a wide variety of cells.

Other experimental results presented in this paper demonstrate that various carbonyl reagents, which have in the past been used to inhibit amine metabolism by amine oxidases believed to contain pyridoxal phosphate as their cofactor [9–11], are capable of interfering with the solvent-extraction of amine metabolites in radiochemical assays for MAO. Thus, considerable caution is required in their use and the interpretation of results obtained with these agents. The magnitude of these effects was dependent upon the carbonyl reagent studied, and also on the substrate used to assay MAO. In addition, conditions were described in which the deamination of 5-HT in the presence of semicarbazide or isoniazid appeared, paradoxically, to be greater than in control samples. This effect occurred when 5-HT deamination was allowed to proceed for times beyond which metabolite production in control assays was no longer linear. These findings may also be due to the properties

of these compounds as carbonyl reagents. Amine metabolism by MAO has been shown to be sensitive to product inhibition by aldehyde metabolites [24], and the accumulation of these products may be one factor responsible for the deviation from linearity of the reaction rate with increasing reaction time. The ability of the carbonyl reagents to form adducts with aldehydes as they are produced in the enzymic reaction may help to protect the enzyme from product inhibition during prolonged reaction times and therefore, result in a less marked slowing with time of the reaction rate in the presence of these compounds, as was seen in the present experiments (Fig. 5). In this way, the greater production of amine metabolites by the enzymic reaction in the presence of the carbonyl reagents may more than offset the reduced extraction of these metabolites, also brought about by these compounds, with the net result that the apparent enzymic activity is greater than controls under these circumstances.

In common with semicarbazide and isoniazid, aminoguanidine also appeared to interfere with metabolite extraction in MAO assays. However, this compound at a concentration of 2×10^{-3} M also appeared capable of inhibiting amine metabolism by MAO directly to some degree, even though it has been suggested that this compound is not an inhibitor of MAO [11]. The concentration of aminoguanidine used in this study was higher than those concentrations (10^{-5} to 10^{-7} M) at which aminoguanidine has generally been reported to act as an inhibitor of the soluble amine oxidases [10, 14]; consequently, the present results may indicate that this compound is a less specific inhibitor of this latter class of enzymes at higher concentrations. Any effects of this agent upon MAO would appear to be totally reversible since additional studies have shown that the inhibition is not time-dependent and, furthermore, can be abolished completely by subjection of aminoguanidine-treated mitochondria to the dilution, washing and re-isolation procedures described earlier in this paper for the experiments involving histamine (unpublished results).

The rationale for using histamine at the beginning of these experiments was that this compound, which is a good substrate for diamine oxidase (DAO), might inhibit any metabolism, by this enzyme, of the other amine substrates studied. This agent did, indeed, inhibit directly the deamination of both 5-HT and benzylamine. However, since we have concluded that mitochondrial MAO appears to be the only significant enzymic activity responsible for deaminating these substrates, it appears that histamine is, instead, capable of inhibiting both type A and type B MAO in rat kidney mitochondria by a preincubation-time-dependent, oxygen-dependent and predominantly irreversible mechanism. The time-dependence and requirement for oxygen may indicate that histamine is being metabolized to an active principle, possibly by an amine oxidase, in the mitochondrial fractions. Histamine is not considered generally to be a substrate for MAO. On the other hand, DAO has been detected recently in rat kidney homogenates by the use of a sensitive radio-isotopic assay to measure histamine degradation, although, in contrast to several other species, this enzyme is present at only exceedingly low levels of activity in this organ [25]. Whether or not such an activity could be present at significant levels in our mitochondrial preparations is not clear. On the other hand, histamine itself may be the inhibitory agent. The irreversible nature of the inhibition observed may be due to the inhibitor being tightly or covalently bound at or near the enzyme active site, or alternatively, a conformational change in enzyme structure, induced by preincubation with histamine, might impair the ability of MAO to bind and metabolize substrates. Clearly, we are unable to distinguish between these and other potential mechanisms on the basis of the present data, but further studies are in progress in an attempt to differentiate between these possibilities. However, these effects are not unique to MAO from rat kidney, because a similar time-dependent inhibition of 5-HT and benzylamine metabolism has been obtained in preliminary studies after preincubation of histamine (2×10^{-3} M) with rat liver mitochondria (unpublished observations).

In conclusion, these studies on the inhibition of rat kidney mitochondrial MAO have provided a number of interesting observations. First, the pattern of substrate specificities for the types A and B MAO activities in this organ differs from that reported for other rat tissues. Second, the interference of carbonyl reagents with the assay of MAO activity is a factor which must be considered when using these agents as potential inhibitors. Finally a time- and oxygen-dependent, irreversible inhibition of MAO activity arising from preincubation of mitochondria with histamine has been discovered, and this deserves further investigation.

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