Occurrence of Newly Synthesized Monoamine Oxidase in Subcellular Fractions of the Rat Liver

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Abstract ☐ The rates of return of monoamine oxidase activity in rat liver mitochondrial and microsomal fractions following inhibition by pargyline or iproniazid were investigated. When these irreversible inhibitors were administered at dosages which produced 100% inhibition, the rate of return of enzyme activity was found to be more rapid in the microsomal fractions than in the mitochondrial fractions. In addition, it was observed that emetine or chloramphenicol inhibited the synthesis of monoamine oxidase but inhibition was not noted until 48 hr. after administration of these compounds.

Keyphrases ☐ Monoamine oxidase, newly synthesized—rat liver ☐ Inhibition, monoamine oxidase—activity return rate ☐ Emetine effect—monoamine oxidase synthesis ☐ Chloramphenicol effect—monoamine oxidase synthesis ☐ UV spectrophotometry—analysis ☐ Fluorometry—analysis

The enzyme monoamine oxidase (MAO) which deaminates a variety of aromatic amines is localized primarily in the mitochondrial fractions of various mammalian tissues (1). This enzyme has been shown to be specifically associated with the outer membrane of the rat liver mitochondria (2) and has been used as a marker for mitochondrial studies. In order to study the rate of synthesis of mitochondria, Barondes (3) determined the rate of regeneration of MAO activity following administration of iproniazid, an irreversible inhibitor of the enzyme (4, 5). However, to date, evidence that MAO is synthesized in the mitochondria has not been obtained. It has been suggested that the small amount of DNA present in mitochondria may be responsible, in part, for the synthesis of mitochondrial proteins (6); but studies have not shown this conclusively and the origin of most mitochondrial proteins is unknown. The studies presented in this paper attempt to show a precursor-pool relationship between the return of MAO activity in microsomes and the return in mitochondria after initial inhibition by irreversible inhibitors of the enzyme. Also, the effects of inhibitors of protein synthesis on the regeneration of MAO activity are examined.

EXPERIMENTAL

Animals used for these studies were adult albino rats (Sprague-Dawley strain) of either sex. Mitochondrial and microsomal fractions were obtained from the livers by the method of Schneider and Hogeboom (7). MAO activity in mitochondrial fractions was determined by the method of Tabor *et al.* (8) and Erwin and Hellerman (9). The mitochondrial fractions were washed twice with 0.25 *M* sucrose and the final mitochondrial pellets were resuspended in approximately 5 ml. of 0.25 *M* sucrose. Of each mitochondrial suspension 0.02 ml. was added to separate cells containing 0.1 ml. of 1% emulsifier[1] and 2.0 ml. phosphate buffer (0.05 *M* KH_2PO_4 adjusted to pH 7.4 with NaOH). After incubation for 3 min. at 37°, 0.05 ml. of 0.05 *M* benzylamine HCl, *meta*-methylbenzylamine HCl, or water was added and the appearance of benzaldehyde or *meta*-methylbenzaldehyde was followed spectrophotometrically at 250 mμ with a spectrophotometer[2] equipped with a recorder.[3] Protein was determined by the biuret method with bovine serum as the standard.

The MAO activity in the microsomal fractions was measured by the method of Weissbach (10) as modified by Erwin and Dietrich (11). To each tube 0.5 ml. microsomal fraction, 0.1 ml. tryptamine (0.1 *M*), and 0.2 ml. aldehyde dehydrogenase [isolated from beef liver by the method of Dietrich, *et al.* (12)], and 0.1 ml. NAD (0.03 *M*) were added and the mixture was allowed to incubate for for 10 min. at 37°. This system permitted the catalytic formation by monoamine oxidase of indoleacetaldehyde from tryptamine with the subsequent oxidation of this compound, by excess NAD and aldehyde dehydrogenase, to indole acetic acid (IAA). The reaction was stopped after 10 min. with 0.2 ml. of 10% zinc sulfate and 0.1 ml. of 1 *N* NaOH. The reaction mixture was then washed with 5 ml. of 1,2-dichloroethane to remove any unreacted tryptamine. One milliliter of the aqueous phase containing the salt (sodium indoleacetate) was then extracted into 15 ml. of 1,2-dichloroethane after reconverting the NaIAA to the free acid (IAA) with 0.1 ml of 6 *N* HCl. Ten milliliters of this extract was reextracted with 3 ml. of 0.5 *M* phosphate buffer, pH 7.4, to yield an aqueous solution of the potassium salt of IAA which was analyzed fluorometrically[4] with primary and secondary filters, 7.54 and 75, respectively.

In order to determine the dose of MAO inhibitors required to produce 100% inhibition of the enzyme activity, rats were injected intraperitoneally with 2.5, 5.0, or 10.0 mg./kg. iproniazid (Aldrich Chemical Co.) or with 5.0, 10.0, or 20.0 mg./kg. pargyline (Abbott Laboratories) in 0.9% saline. Three rats were used for each dosage of the inhibitors and five control animals received an equal volume of 0.9% saline. After 24 hr. the animals were sacrificed and the livers excised. Monoamine oxidase activity in the mitochondrial fractions was determined as above and the percent inhibition calculated for the various dosages.

In the studies concerning the rate of return of MAO activity in mitochondrial and microsomal fractions after administration of iproniazid or pargyline, each of twelve rats were given 10.0 mg./kg. iproniazid and a similar number of animals were injected with 20.0 mg./kg. pargyline. Two animals from each group were sacrificed at 12-hr. intervals and the MAO activity in the mitochondrial and microsomal fractions was determined as described above. Control studies were performed with four animals which had received saline injections in place of the MAO inhibitors.

Inhibition of MAO synthesis by emetine or chloramphenicol was determined by initially treating twelve animals with 20.0 mg./kg. pargyline. Then, one-half of these animals was given 3 mg./kg. of emetine HCl (City Chemical), and the other received 50 mg./kg. chloramphenicol (Parke-Davis and Co.). All injections were given intraperitoneally. After 12 hr. one rat from each group was sacrificed and the liver mitochondria analyzed for MAO activity. The remaining animals received a similar dose of emetine or chloramphenicol every 24 hr.; thus, the dosage of these drugs was maintained in the last rats for five days. Four control animals were injected with saline in place of the initial dose of pargyline.

RESULTS

As shown in Table I, rat liver mitochondrial MAO was inhibited approximately 80% by 2.5 mg./kg. iproniazid or 5.0 mg./kg.

[1] Lubrol-wx, I.C.I./Organics/Inc., Providence, R. I.

[2] Beckman DB.
[3] Graphicorder 10.
[4] Turner, model 111.

Table I—Effect of Various Doses of Iproniazid or Pargyline on Rat Liver Mitochondrial Monoamine Oxidase

Drug	Dose, mg./kg.	% Inhibition[a]
Iproniazid	2.5	80
Iproniazid	5.0	92
Iproniazid	10.0	100
Pargyline	5.0	75
Pargyline	10.0	83
Pargyline	20.0	100

[a] Activity of monoamine oxidase in the mitochondrial fractions was determined as described in the text. Experimental procedures are as described in the text.

pargyline. However, the dosages of iproniazid or pargyline found to give 100% inhibition of the enzyme were considerably higher, *i.e.*, 10.0 mg./kg. and 20.0 mg./kg., respectively. Therefore, these dosages were employed in the following experiments.

Data presented in Fig. 1 show that MAO activity in mitochondria returned to 50% of the control value 3.5 days and 3.3 days after inhibition by iproniazid or pargyline, respectively. After total inhibition of monoamine oxidase activity by iproniazid or pargyline the rate of return in activity in mitochondria was gradual at first, reaching approximately 15 to 20% after 60 hr. However, in the next 60 hr. activity had increased to approximately 80% of the control value.

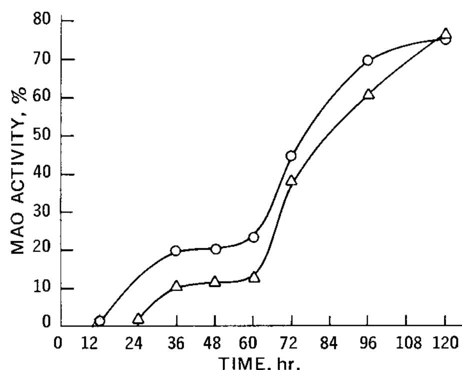

Figure 1—*Return of rat liver mitochondrial monoamine oxidase activity after treatment with iproniazid* (△-△), *or pargyline* (○-○).

As shown in Fig. 2, in iproniazid-treated animals the rate of return of MAO activity in the microsomal fractions was rapid between 48 and 60 hr., reaching 77% at 60 hr., after which time activity decreased slightly. After inhibition by pargyline the MAO activity in the microsomal fractions rose to only 10% by 48 hr. followed by a rise in activity, to 28%, between 48 and 72 hr., after which a gradual decrease to 23% at 120 hr. was observed.

Since Grollman (13) demonstrated that emetine inhibited protein synthesis in mammalian tissues, the effects of this alkaloid on MAO synthesis was investigated. As shown in Fig. 3, during the first

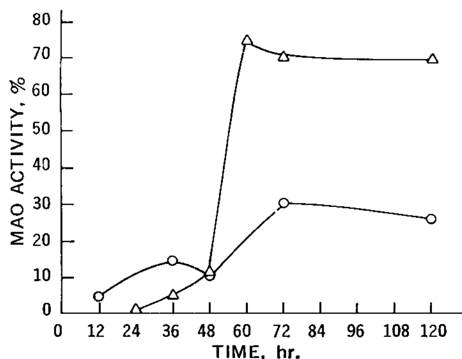

Figure 2—*Return of rat liver microsomal monoamine oxidase activity after treatment with iproniazid* (△-△), *or pargyline* (○-○).

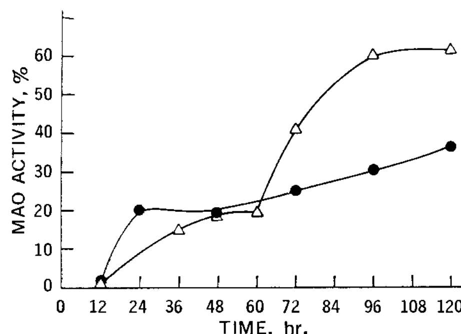

Figure 3—*Return of rat liver mitochondrial monoamine oxidase activity after treatment with pargyline* (△-△), *treatment with pargyline plus emetine* (●-●). *See text for details.*

60 hr., the rate of return of MAO activity in liver mitochondria isolated from rats receiving emetine every 24 hr. paralleled the rate of return of MAO activity in animals not receiving emetine. However, after 60 hr., emetine produced a marked decrease in the rate of return of MAO activity. These results indicate that emetine inhibits MAO synthesis only after a lag period of approximately 48 to 60 hr.

Monoamine oxidase activity in livers of animals receiving chloramphenicol was comparable to that of saline treated animals with an average of 86% activity throughout the 5-day study. The data presented in Fig. 4, show that when chloramphenicol was administered to rats after treatment with pargyline, the return of MAO activity paralleled the return of MAO activity in animals not receiving chloramphenicol for the first 48 hr. After this time, no substantial return was observed in the chloramphenicol-treated animals, indicating total inhibition of MAO synthesis. The activity of the mitochondrial fractions remained between 32.5 and 33.5% of the control value for the next 48 hr. after which time a slight increase to 36.5% was seen. However, the rate of return during this last period of the assay was markedly less than the control rate.

DISCUSSION

It was observed that the rates of return of MAO activity in the mitochondrial and microsomal fractions from iproniazid-treated rats were parallel throughout the first 48 hr. At 48 hr., a plateau was reached in the rate of return of MAO activity in the mitochondrial fractions while activity rapidly increased in the microsomal fractions. At the height of the increase in MAO activity in the microsomal fractions, *i.e.*, 60 hr., the plateau seen in the rate of return of activity in the mitochondria ended and a rapid increase in activity ensued. The microsomal activity began decreasing slightly at this point. Results obtained with pargyline-treated rats were qualitatively similar to those observed with iproniazid-treated animals. From these data, it may be postulated that a precursor-pool relationship between the microsomal and mitochondrial MAO levels exists, whereby MAO originates in the microsomes and is transferred to the mitochondria.

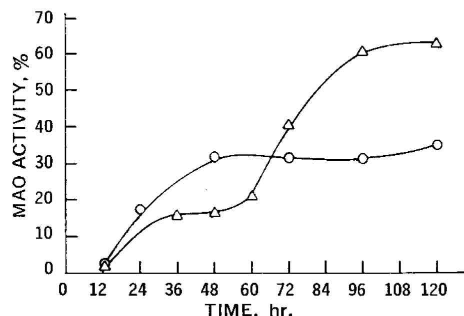

Figure 4—*Return of rat liver mitochondrial monoamine oxidase activity after treatment with pargyline* (△-△) *or treatment with pargyline and chloramphenicol* (○-○). *See text for details of procedure.*

The marked decrease in the rate of return of monoamine oxidase activity approximately 48 hr. following inhibition with pargyline and treatment with emetine, may be the result of the partial inhibition of monoamine oxidase synthesis by emetine. According to the mechanism proposed by Grollman (13), protein synthesis is inhibited by emetine at the site of peptide bond formation, a late step in the synthesis sequence. It seemed relevant to use an inhibitor of protein synthesis which acts early in the sequence, in order to possibly predict some properties of the *m*-RNA responsible for the direction of MAO synthesis. Chloramphenicol was used for this purpose, since it is known from the work of Weisberger (14) that chloramphenicol inhibits protein synthesis at the site of the attachment of the *m*-RNA to the ribosomes. The results of this study showed a complete inhibition of monoamine oxidase synthesis after 48 hr. There was a decrease in the rate of MAO synthesis approximately 48 hr. following teatment with either emetine or chloramphenicol suggesting a constant lag time for the inhibition of protein synthesis in the rat liver. Currently, further studies on the synthesis of MAO and other mitochondrial proteins are in progress.

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Synthesis of 2-Ethyl-3-methyl-5-morpholinomethyl-4-keto-4,5,6,7-tetrahydrothionaphthene

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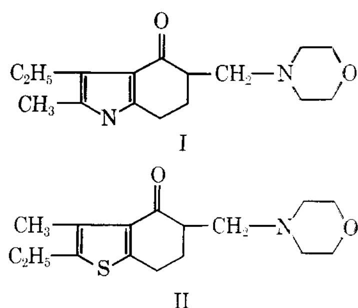
Abstract □ The title compound was prepared and evaluated for central nervous system, cardiovascular, autonomic, endocrine, anti-inflammatory, antiallergic, and metabolic activities. No significant activity was noted.

Keyphrases □ 2-Ethyl-3-methyl-5-morpholinomethyl-4-keto-4,5,6,7-tetrahydrothionaphthene—synthesis □ GLC—analysis □ IR spectrophotometry—structure □ NMR spectroscopy—structure

Previously reported work (1) indicated that the Mannich bases of 4-keto-4,5,6,7-tetrahydrothionaphthene possessed some degree of biological activity. The marked activity (2) of molindone (I) led to the synthesis of the closely related bicyclic thiophene (II).



The preparation of IX was carried out according to procedures previously described in the literature (3). The separation of IV and V was accomplished on a 91.44-cm. (36-in.) stainless steel spinning band column and the purity of the fractions determined *via* GLC. Yields of IV–IX were comparable to those described in the literature.

Compound II was screened for CNS, cardiovascular, autonomic, endocrine, anti-inflammatory, antiallergic, and metabolic activities, however, no significant activity was noted.