EFFECT OF TRIS (HYDROXYMETHYL) AMINOMETHANE ON AMINE OXIDASE ACTIVITY IN DOG BRAIN, LIVER AND SERUM AND IN HUMAN PLACENTA

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Abstract—The effect of Tris (hydroxymethyl) aminomethane on mitochondrial monoamine oxidase (MAO) activity in dog brain and liver and in human placenta was studied and the results obtained were compared with results on dog serum amine oxidase. With benzylamine as substrate, Tris did not inhibit mitochondrial MAO activity in these preparations, whereas it inhibited amine oxidase activity in a serum preparation. However, with tyramine, 5-hydroxytryptamine or β-phenylethylamine as substrate, Tris inhibited MAO activity in all these preparations and its mode of inhibition was found to be non-competitive and reversible. The inhibition by Tris of MAO activity in these preparations was not due to decrease in the extent of extraction of aldehydes produced during the enzyme reaction. Moreover, increase in the oxygen tension did not change the extent of inhibition of MAO activity by Tris. From these results, it is concluded that with benzylamine as substrate, there is a remarkable difference in the effects of Tris on amine oxidase activity in mitochondrial and serum preparations. This difference in the inhibitions of mitochondria and serum is discussed.

There is considerable evidence that mitochondrial monoamine oxidase (MAO) [monoamine: O₂ oxidoreductase (deaminating) EC 1.4.3.4] in various tissues from many species exists in more than one form (1, 2).

Tris (hydroxymethyl) aminomethane (Tris) has usually been used as a buffer in biochemical studies and is used clinically for correction of acidosis.

Recently, Ikeno et al. (3) reported that Tris inhibited amine oxidase activity in dog serum with benzylamine as substrate, and Yasunobu and Oi (4) reported that it inhibited MAO activity in beef liver mitochondria towards benzylamine. However, Fowler et al. (5), found that Tris inhibited MAO activity in rat liver mitochondria towards various monoamine substrates, but not towards benzylamine. Thus, different results were obtained in mitochondrial and serum preparations on the effects of Tris on MAO activity with benzylamine as substrate.

In the present work, we studied the effect of Tris on mitochondrial MAO activity in various tissues (dog brain and liver and human placenta) with 5-hydroxytryptamine (5-HT), tyramine, β-phenylethylamine (PEA) and benzylamine as substrates. For comparison we made similar studies on dog serum amine oxidase. We also attempted to determine the mechanism of the inhibitory
action of Tris on MAO in these preparations.

MATERIALS AND METHODS

Chemicals: Labelled 5-hydroxytryptamine bioxalate (44 mCi/m mol), tyramine hydrochloride (56.2 mCi/m mol) and β-phenylethylamine hydrochloride (48.25 mCi/m mol) were purchased from New England Nuclear, Boston, Mass., U.S.A. Benzylamine hydrochloride (56 mCi/m mol) was purchased from the Radiochemical Centre in England. All other chemicals used were of the highest grade commercially available.

Enzyme preparation: Dogs were anesthetized with pentobarbital, exsanguinated and the brain and liver rapidly removed. Human placenta were obtained after normal deliveries and the tissues were weighed and cut into small pieces. These tissues were individually homogenized, first in a Waring Blender and then in a glass homogenizer with 9 volumes of ice-cold 0.25 M sucrose (liver) or 0.32 M sucrose (brain) containing 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 600 x g for 10 min, and the resulting supernatants were collected and recentrifuged at 8,500 x g for 20 min. The precipitates were suspended in sucrose solution and again centrifuged at 8,500 x g for 20 min. The precipitates were then suspended in 5 volumes of 0.01 M phosphate buffer (pH 7.4) and used as mitochondrial preparations.

Partially purified serum amine oxidase was prepared as described previously (3). Briefly, dog blood was collected, left for 30 min and then centrifuged at 3,000 x g for 10 min to separate the serum. Saturated ammonium sulfate solution was added to this serum preparation to give a final concentration of 33% and the preparation was centrifuged at 4,700 x g for 20 min. Then saturated ammonium solution was again added to the supernatant to give a final concentration of 66% and the precipitate was collected by centrifugation at 4,700 x g for 20 min. This precipitate was dissolved in a small volume of 3.3 mM phosphate buffer (pH 7.4), dialyzed for 16 hr against a large volume of 1.0 mM phosphate buffer (pH 7.4) with two changes of the outer fluid and used as the preparation of dog serum amine oxidase.

Assay of MAO activity: Enzyme activity was determined at 37°C and pH 7.4 by the radiometric assay method of Wurtman and Axelrod (6). The reaction mixture, containing enzyme solution, 0.1 M phosphate buffer (pH 7.4) and various concentrations of Tris-HCl adjusted also to pH 7.4 in a total volume of 275 µl was preincubated at 37°C for 20 min. Then 25 µl of substrate solution at a concentration of about twice the Km value for each was added for estimation of enzyme activity. The incubation was carried out at 37°C for 20 min and then 200 µl of 2N HCl was added to stop the reaction. The enzyme activity was found to be proportional to the incubation period and to the amount of enzyme preparation used. Then metabolites were extracted with ether (with tyramine or 5-HT as substrate) or toluene (with benzylamine or PEA as substrate), by vigorous shaking and the radioactivity in the organic layer was estimated with a Packard Tri-Carb Liquid Scintillation Spectrometer. The concentrations of substrates used were approximately twice the Km values.

Protein concentrations were estimated by the modified biuret method (7) with bovine serum albumin as a standard.

RESULTS

Effect of Tris on MAO activity in various mitochondrial preparations: The effect of Tris on mitochondrial MAO activity in dog brain is shown in Fig. 1A. At concentrations of 10^-6 M to 10^-3 M, it had no effect on MAO activity with 5-HT, tyramine, PEA or benzylamine as substrate. But, at 10^-2 M, it inhibited the activity with tyramine, 5-HT and
PEA by 20, 19 and 14%, respectively (Fig. 1A). On the other hand, 10⁻¹ M Tris did not inhibit MAO activity with benzylamine as substrate, but inhibited the activity with other substrates about 60–80% (Fig. 1A). The effect of oxygen tension on inhibition of MAO activity in dog brain mitochondria with tyramine, 5-HT and benzylamine as substrates was also studied and the results are shown in Fig. 1B. The effects of Tris on MAO activity were similar in assay mixtures exposed

![Graph](image1.png)

**Fig. 1A and B.** Inhibition of MAO activity in dog brain towards tyramine, 5-HT, benzylamine and PEA by various concentrations of Tris. Mitochondrial preparation (0.5 mg of protein) from dog brain was preincubated with various concentrations of Tris-HCl at 37°C for 20 min. Labelled substrate was then added to estimate the remaining MAO activity under an atmosphere of air (A) or oxygen (B) with 0.3 mM tyramine (●—●), 0.5 mM 5-HT (△—△), 0.3 mM benzylamine (×—×) or 0.02 mM PEA (□—□) as substrate. Activity is expressed as a percentage of the control MAO activity estimated in the absence of Tris-HCl. The control MAO activities with these substrates were 0.52 (tyramine), 0.51 (5-HT), 1.12 (benzylamine) and 0.01 (PEA) n mol product formed/mg protein/min at 37°C, pH 7.4. Each point is the mean of duplicate determinations.

![Graph](image2.png)

**Fig. 2A-C.** Inhibition of MAO activity towards tyramine, benzylamine, 5-HT and PEA by various concentrations of Tris. Mitochondrial preparations from dog liver (A; 0.28 mg of protein), human placenta (B; 0.35 mg) and dog serum (C; 1.05 mg) were preincubated as described in the legend to Fig. 1. The remaining MAO activity was estimated under an atmosphere of air. In Fig. 2C, 5-HT was not used as substrate for assay of the activity. The control MAO activities with tyramine, benzylamine, 5-HT and PEA were: 5.50, 11.19, 0.17 and 0.06 (rat liver); 1.46, 1.08, 3.53 and 0.07 (human placenta); 0.003, 3.24 and 0.004 (dog serum) n mol product formed/mg protein/min at 37°C, pH 7.4, respectively. The symbols for substrates used are as in Fig. 1.
to 100% oxygen or to air during the reaction (Fig. 1B).

The effect of Tris on mitochondrial MAO activity in dog liver was similar to that in brain (Fig. 2A). At a concentration of $10^{-3}$ M, Tris did not inhibit MAO activity towards tyramine, 5-HT or PEA, but at a concentration of $10^{-2}$ M it caused 34 and 49% inhibitions of tyramine and 5-HT oxidation, respectively. At a concentration of $10^{-1}$ M, Tris did not inhibit MAO activity with benzylamine as substrate in this mitochondrial preparation (Fig. 2A). Similar results were obtained with human placental mitochondria to those obtained with dog brain and liver mitochondria (Fig. 2B).

Results on the effect of Tris on amine oxidase activity in dog serum are shown in Fig. 2C. Inhibition of benzylamine oxidation in the serum differed from that in mitochondrial MAO preparations. At concentrations of $10^{-2}$ M and $10^{-1}$ M, Tris caused 62 and 94% inhibitions, respectively, of the activity towards tyramine, but with PEA as substrate, it caused 21 and 93% inhibition, respectively. At the same concentrations, it caused 81 and 91% inhibitions, respectively, of the activity with benzylamine. Thus, with benzylamine as substrate, the effects of Tris on the enzyme activity in the mitochondria and serum were different.

The modes of inhibition by Tris of the MAO activities of dog brain and human placental mitochondria and dog serum were studied by Lineweaver-Burk plots. Tris was found to cause non-competitive inhibition of tyramine and 5-HT oxidations in mitochondria and of tyramine and benzylamine oxidations in the serum preparation (Figs. 3–5).

Lineweaver-Burk plots of MAO activity in dog brain towards tyramine under an atmosphere of 100% oxygen in the presence of Tris (33 mM) also indicated that Tris caused non-competitive inhibition (Fig. 6).

Reversibility of inhibition by Tris of MAO activity in dog brain, human placenta and

dog brain

![Fig. 3](image-url)

Fig. 3. Lineweaver-Burk plots of inhibition of MAO activity in dog brain towards tyramine and 5-HT by Tris. Dog brain mitochondrial preparation (0.5 mg of protein) was preincubated with 10 mM (△—△) or 33 mM (□—□) Tris-HCl at 37°C for 20 min. Control samples were preincubated at 37°C for 20 min without Tris (○—○). MAO activity was then estimated in air with various concentrations of tyramine (left; concentration ranges, 83.3–500 μM) or 5-HT (right; 40–200 μM) as substrates. The abscissa and the ordinate show 1/(substrate concentration) and 1/DPM×10⁻⁴, respectively.
**dog serum:** The reversibility of the inhibition by Tris of dog brain and human placental mitochondrial MAO activity was studied by experimentation with dialysis, and the results are shown in Table 1. A mixture of the mitochondrial preparation from dog brain, 0.1 M Tris-HCl adjusted to pH 7.4, and 0.01 M phosphate buffer (pH 7.4) was

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**human placenta**

![Lineweaver-Burk plots](image)

**Fig. 4.** Lineweaver-Burk plots of inhibition of MAO activity in human placenta towards tyramine and 5-HT by Tris. The experiments were carried out as described in the legend to Fig. 3, but with human placental mitochondria (0.35 mg of protein) as enzyme preparation.

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**dog serum**

![Lineweaver-Burk plots](image)

**Fig. 5.** Lineweaver-Burk plots of inhibition of amine oxidase activity in dog serum towards tyramine and benzylamine by Tris. The experiments were carried out as described in the legend to Fig. 3, but with dog serum (1.05 mg of protein) and various concentrations of tyramine (left: 0.83–5.7 μM) and benzylamine (right: 83.3–500 μM) as substrates. The symbols for the concentrations of Tris-HCl are as in Fig. 3. The ordinate for the left figure shows 1/DPM×10^-3, but for the right figure it shows 1/DPM×10^-4.
dialyzed for 16 hr in a cold room against a large volume of 1.0 mM phosphate buffer (pH 7.4). As a control, a mixture of the mitochondria, phosphate buffer and distilled water, instead of Tris, was dialyzed under the same conditions. Similar mixtures, with and without Tris, were also placed for 16 hr in a cold room without dialysis as controls. The preparation containing 0.1 M Tris, which had been placed in a cold room for 16 hr, showed 69, 73 and 80% of the control MAO activities (without Tris) with tyramine, 5-HT and PEA, respectively, as substrates. After dialysis, the activities of the mixture containing Tris were 99, 113 and 107%, respectively, of the control values, indicating that Tris reversibly inhibited MAO activity (Table 1). Similar experiments were carried out with human placenta and dog serum. The results in Table 1 also show that Tris reversibly inhibited the enzyme activity in these preparations.

**Fig. 6.** Lineweaver-Burk plots of inhibition of MAO activity in dog brain towards tyramine by Tris. The experiments were carried out as described in the legend to Fig. 3, under an atmosphere of 100% oxygen with various concentrations (83.3 μM-0.5 mM) of tyramine as substrate. The concentration of Tris-HCl was 33 mM (□⋯⋯□). The ordinate shows 1/DPM×10^-4.

**Table 1.** Effect of dialysis on inhibition of MAO activity by Tris. The experiments were carried out as described in the text, using dog brain (B), human placenta (P) and dog serum (S) preparations and tyramine (0.3 mM for brain, 0.2 mM for placenta and 0.01 mM for serum), 5-HT (0.5, 0.5 and 0.1 mM for brain, placenta and serum, respectively), benzylamine (0.3, 0.5 and 0.3 mM for brain, placenta and serum, respectively) and PEA (0.02, 0.1 and 0.05 mM for brain, placenta and serum, respectively) as substrates. The enzyme activity is expressed as a percentage of the control in the absence of Tris-HCl (−). The control MAO activities with these substrates are indicated in legends for Figs. 1A, 2B and 2C. Each value is the mean of duplicate determinations.

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<th>Dialysis</th>
<th>Tris</th>
<th>% of control MAO activity</th>
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<td>99 117 98 113 111 116 100 133 91 107 117</td>
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B: dog brain  P: human placenta  S: dog serum
shown in Table 2, the radioactivity extracted after incubation (in the absence of Tris) was similar to that extracted from the preparation treated with 33 mM Tris just before stopping the reaction. Thus, Tris does not affect the extent of extraction of the products from the substrates during the enzyme reaction.

**Table 2.** Effect of Tris on the rate of extraction of MAO metabolites. The enzyme activity in dog liver and serum was estimated in the absence of Tris-HCl (--) or in the presence of 33 mM Tris-HCl added just before stopping the enzyme reaction. Activity, expressed as a percentage of the control activity in the absence of Tris-HCl, was estimated with tyramine (0.5 mM for liver and 0.1 mM for serum), 5-HT (0.25 and 0.1 mM for liver and serum, respectively), benzylamine (0.2 and 0.3 mM for liver and serum, respectively) and PEA (0.02 and 0.05 mM for liver and serum, respectively) as substrates. The control MAO activities with these substrates are indicated in legends for Figs. 1A, 2B and 2C. Each value is the mean of duplicate determinations.

<table>
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<th>Tyramine</th>
<th>5-HT</th>
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<td>33 mM</td>
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Inhibited amine oxidase activity towards all the substrates, including benzylamine. The latter finding shows that the lack of inhibition of benzylamine oxidation by mitochondrial preparations was not due to their contamination with serum amine oxidase, which is highly active towards benzylamine. Thus, the effects of Tris on benzylamine oxidation in mitochondria and the serum were different. The results obtained for mitochondrial preparations are in agreement with those of Fowler et al. (5) and of Browne et al. (8), who both found that Tris did not inhibit benzylamine oxidation in rat liver mitochondria. However, our results do not agree with those of Yasunobu and Oi (4), who reported that Tris inhibited mitochondrial MAO activity of bovine liver towards benzylamine. The difference in the inhibitions by Tris of mitochondrial and serum MAO towards benzylamine may be due to differences in the co-enzymes of these preparations, FAD for mitochondrial MAO (1, 9), but presumably, pyridoxal phosphate for serum amine oxidase (10), or to differences in the conformation of the enzyme proteins.

**DISCUSSION**

This study showed that Tris inhibited mitochondrial MAO activity towards tyramine, 5-HT and PEA, but not benzylamine; in contrast with a dog serum preparation it inhibited amine oxidase activity towards all the substrates, including benzylamine. The latter finding shows that the lack of inhibition of benzylamine oxidation by mitochondrial preparations was not due to their contamination with serum amine oxidase, which is highly active towards benzylamine. Thus, the effects of Tris on benzylamine oxidation in mitochondria and the serum were different. The results obtained for mitochondrial preparations are in agreement with those of Fowler et al. (5) and of Browne et al. (8), who both found that Tris did not inhibit benzylamine oxidation in rat liver mitochondria. However, our results do not agree with those of Yasunobu and Oi (4), who reported that Tris inhibited mitochondrial MAO activity of bovine liver towards benzylamine. The difference in the inhibitions by Tris of mitochondrial and serum MAO towards benzylamine may be due to differences in the co-enzymes of these preparations, FAD for mitochondrial MAO (1, 9), but presumably, pyridoxal phosphate for serum amine oxidase (10), or to differences in the conformation of the enzyme proteins.

High concentrations of various buffers were reported to cause partial inhibition of
MAO activity towards various amine substrates, but, unlike the case with Tris, the extent of inhibition by these buffers did not depend on the substrate used, indicating that the inhibitions were non-specific (11).

Lewinsohn et al. (12-14), recently reported evidence for existence of a clorgyline-insensitive, but semicarbazide-sensitive amine oxidase in many tissues of some species which can oxidize benzylamine, but is distinct from mitochondrial MAO (type A MAO and type B MAO) and benzylamine oxidase, which is thought to be present only in plasma. Thus, another possible explanation for the differences in the results on benzylamine oxidation might be that this oxidation is predominantly catalyzed by the benzylamine oxidase in mitochondria, and that Tris does not inhibit this enzyme activity. Indeed, the presence of this enzyme, which is relatively insensitive to clorgyline (10^-3 M), but which can oxidize benzylamine, was demonstrated in human placental mitochondria in this study. As shown in this study, however, Tris also inhibited this enzyme activity. Moreover, the proportion of this activity, to the total activity for oxidation of benzylamine, is small (about 20%) in the mitochondria with benzylamine as substrate. Thus, the proportion of the mitochondrial MAO activity that is relatively insensitive to Tris was probably not due to benzylamine oxidase in the mitochondria.

Although human serum amine oxidase apparently did oxidize 5-HT (15), as shown in this study, dog serum MAO catalyzed the oxidation of benzylamine, tyramine and PEA (Fig. 2C), as well as 5-HT (Tables 1 and 2). These oxidations might be due to contamination of the serum preparation with mitochondria, presumably originating from platelets containing type B MAO only (16, 17). But this possibility was excluded by the fact that the MAO activity towards benzylamine in serum was inhibited by Tris, whereas, that in mitochondrial preparations was usually not inhibited.

The mode of inhibition of MAO activity by Tris was found to be non-competitive and reversible towards the substrates tested. This is in accordance with the results of Fowler et al. (5), but in contrast with those of Yasunobu and Oi (4), who reported that the inhibition was uncompetitive. For non-competitive inhibition, Tris must act on a site(s) other than the substrate binding site(s) on the enzyme in dog brain, liver and serum and human placenta (Figs. 3-5). The site of action must also differ from the binding site(s) of the second substrate for MAO, oxygen, since the extent and mode of inhibition by Tris were similar in reaction mixtures under oxygen and air (Figs. 1A, B and 6).

There are several possible mechanisms for the inhibitory action of Tris towards MAO activity, some of which have already been discussed by other investigators (5): Tris might be considered to decrease the affinity of MAO for oxygen, its second substrate, by disturbing the binding in some way and thus decreasing the activity. This possibility is excluded, as discussed above, by the finding that increase in the oxygen tension did not affect the degree or mode of inhibition by Tris. Another possibility is that Tris might interfere with the extraction of the reaction products into the organic solvent. However, this seems unlikely from the present work. A third possibility is that Tris might form complexes with substrates in the assay medium, decreasing the substrate concentrations, and thus decreasing the activity. Mahler (18) reported that Tris may form a complex with a substrate containing a carbonyl group. This possibility was also suggested by Ogilvie and Whitaker (19) for inhibition of homoserine dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase activity; that is, Tris might form reversible
complexes with aspartic-β-semialdehyde and glyceraldehyde-3-phosphate, the substrates for these two enzymes. But the substrates used in this study, monoamines, do not have a carbonyl group, and, moreover, Tris did not produce complexes with aldehydes formed from the amine substrates during the enzyme reaction. Therefore, it is unlikely that Tris inhibited MAO activity by formation of complexes with either the substrates or products. Fourth, an anion (chloride ion) from the Tris salt, as used in this study, may possibly inhibit MAO activity (20). However, such was excluded by showing that different Tris salts caused similar extents of inhibition of rat liver MAO (5). Fifth, Tris might act as a substrate for MAO (19). However, as already discussed above, this is also unlikely, since it caused non-competitive inhibition of the oxidation of amine substrates. Lastly, Tris might affect the enzyme molecule. There have been many reports on the existence of at least two different types of mitochondrial MAO, termed type A MAO and type B MAO, demonstrated by differences in their sensitivities to MAO inhibitors, such as clorgyline and deprenyl (21-25). This classification into two types is not based on differences in their substrate specificities (26-29). However, various findings indicate that Tris does not have a selective effect on either of the two types of MAO in dog brain, liver and human placenta. The relative proportions of the two types of MAO activity in dog brain, liver and human placental mitochondria differ considerably (30-32). Yet, in spite of this difference in the proportions of the two types, Tris caused almost equal inhibitions of the oxidations of tyramine, a substrate for both types, 5-HT, a substrate for type A MAO, and PEA, a substrate for type B MAO at relatively low concentrations (27-29). Moreover, oxidation of benzylamine, which is a substrate for type B MAO only (24), was not inhibited by Tris. This lack of a selective action of Tris on one type of MAO may be because Tris acts on a site(s) other than the sites for binding the first substrate, amines and the second substrate, oxygen, as discussed above.

The pharmacological actions of Tris on various animal organs, e.g., its reversible potentiation of the effect of norepinephrine on the rabbit ear artery (33), might be in part due to the reversible inhibition of MAO activity demonstrated in this study. However, studies on the precise mechanism of the action of Tris on animal organs are required before any conclusion can be reached.

REFERENCES


