EFFECTS OF LOCAL ANESTHETICS ON MONOAMINE OXIDASE, AND THEIR MEMBRANE EFFECTS

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Abstract—The effects of various local anesthetics on rat brain and liver monoamine oxidase (MAO) and their antihemolytic and local anesthetic effects were studied. All local anesthetics tested at $1 \times 10^{-7}$ M to $1 \times 10^{-3}$ M inhibited MAO activity in rat liver mitochondria with 5-hydroxytryptamine (5-HT) as substrate. The order of potency was tetracaine > procaine > dibucaine > lidocaine > prilocaine. Tetracaine and procaine inhibited 5-HT oxidation much more than 3-phenylethylamine (PEA) oxidation. Dibucaine inhibited PEA oxidation as much as 5-HT oxidation. Inhibition of MAO by local anesthetics other than dibucaine was reversible. Tetracaine and procaine inhibited 5-HT oxidation competitively, whereas dibucaine inhibited it non-competitively. Antihemolytic effects were observed with dibucaine and tetracaine at concentrations of $6 \times 10^{-5}$ M and $1 \times 10^{-4}$ M, respectively. The order of surface anesthetic potencies was dibucaine > tetracaine > prilocaine > lidocaine > procaine. These results suggest that the inhibition of MAO activities by local anesthetics depends on both electrostatic and hydrophobic interactions between these drugs and enzyme-associated phospholipids or the hydrophobic regions of proteins.

Biological membranes have been postulated to have a fluid mosaic structure (1). The essential feature of this model is that at physiological temp., the lipids of functional membranes are in a mixture of fluid and solid states (2). The activities of many membrane-bound enzymes may be modulated by changes in the fluidity of the lipid bilayer (3–5). It is widely accepted that two functionally different forms of monoamine oxidase [MAO, EC 1.4.3.4] which are localized in the outer mitochondrial membrane are intrinsic membrane-bound flavoproteins (6). 5-Hydroxytryptamine (5-HT) is preferentially deaminated by type A MAO, whereas 3-phenylethylamine (PEA) is preferentially deaminated by type B MAO (7, 8).

Recently, it has been demonstrated that the activity of type A MAO is strongly modulated by lipid-protein interactions occurring in the hydrocarbon core region proximal to the membrane hydrophilic surface, while the activity of type B MAO does not appear to be affected by the fluidity of the bulk membrane lipid (9).

Local anesthetics possess the ability to increase the fluidity of lipid bilayers, and it has been suggested that this property is related to the phenomenon of anesthesia (10, 11), whereas Eyring et al. (12) have argued in favor of a direct interaction between anesthetics and proteins.

In this work we investigated the relationship between MAO inhibition and the effects...
of local anesthetics on membranes.

**MATERIALS AND METHODS**

Preparations of rat brain homogenate and liver mitochondrial MAO: Male Wistar rats weighing 150–200 g were killed by decapitation, and their brains and livers were rapidly removed and chilled. The tissues were weighed and homogenates were prepared in 3 volumes of 0.25 M sucrose containing 0.01 M Tris-HCl buffer (pH 7.4). Brain homogenates were stored at −20°C until use. Liver homogenates were centrifuged at 600×g for 10 min at 4°C, the supernatant was recentrifuged at 8,500×g for 20 min at 4°C, and the pellet was suspended in sucrose Tris buffer and recentrifuged. The pellet (crude mitochondrial fraction) was suspended in 3 volumes of 0.1 M Tris-HCl buffer (pH 7.4) and used as the MAO preparation. Protein concentrations were determined by the modified biuret method (13) with bovine serum albumin as a standard.

**Assay of MAO activity:** MAO activity was determined at 38°C by the radiometric assay of Wurtman and Axelrod (14). The incubation mixture contained 25 μl of MAO preparation and 25 μl of local anesthetic in a total volume of 300 μl of 0.01 M phosphate buffer (pH 7.4), and the reaction was started by adding 25 μl of 14C-5-hydroxytryptamine (5-HT) (1 μCi/ml) or 14C-β-phenylethylamine (PEA) (1 μCi/ml). Incubations were carried out for 20 min at 38°C, and reactions were stopped by adding 200 μl of 2 M HCl. The mixture was extracted by shaking it with 6 ml of ether for 15 sec when 5-HT was used as substrate or with 6 ml of toluene for 20 min when PEA was used as substrate. Then 4 ml of the organic layer was mixed with 6 ml of Aquasol as the scintillation liquid, and radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer. Activity was expressed as disintegrations per min (DPM).

**Inhibition of hypotonic hemolysis of rat erythrocytes:** Rat blood was drawn from the vena cava into a heparinized syringe. The erythrocyte fragility curve was constructed by suspending 50 μl of blood in 5 ml of hemolyzing medium consisting of 154 mM NaCl and 11 mM phosphate buffer, pH 7.4. The osmolarity of this solution was equivalent to that of a 1% NaCl solution (342 mOs/l). The stock solution was diluted with distilled water, and the osmolarity was adjusted to between 291 mOs/l (equivalent to 0.85% NaCl solution) and 68 mOs/l (equivalent to 0.2% NaCl solution). After incubation for 1 hr in the dark at 37°C, the solution was centrifuged at 700×g for 5 min, and the optical density of the hemoglobin in the supernatant solution was measured at 540 nm with a UV-visible spectrophotometer. The osmolarity which induced 70% hemolysis was about 137 mOs/l (equivalent to 0.4% NaCl solution). This concentration was used to measure the effect of drugs.

**Surface anesthetic effects on rabbit cornea:** Surface anesthetic activity was estimated by applying two drops of drug solution to the conjunctival sac of a rabbit eye and measuring the blink reflex at 5, 10, 15, 20, 30, 40, 50 and 60 min.

**RESULTS**

**Effects of local anesthetics on MAO activity in brain homogenate:** The effects of tetracaine, procaine, dibucaine, lidocaine and prilocaine on MAO activity in a brain homogenate were investigated with 5-HT and PEA as substrates (Fig. 1A, B). All local anesthetics tested in this experiment at concentrations of 1×10⁻⁶ M to 1×10⁻³ M inhibited brain MAO activities with 5-HT as substrate. Tetracaine was the most potent local anesthetic tested, its IC₅₀ value being 1×10⁻⁶ M. Procaine was also potent and its IC₅₀ value was 2×10⁻⁵ M. Dibucaine inhibited MAO activity about 60% at a concentration
Fig. 1. Effects of several local anesthetics on MAO activities in rat brain and liver. Brain homogenate (A, B: 2.1 mg or 1 mg of wet weight) and liver mitochondria (C, D: 160 μg or 80 μg of protein) were incubated at 37°C for 20 min in the presence of local anesthetic in a total volume of 300 μl of phosphate buffer (0.01 M, pH 7.4). 5-HT was used at 1.5 x 10⁻⁴ M and PEA at 0.2 x 10⁻³ M. Experiments were carried out in duplicate under an atmosphere of air. Procaine; - - - prilocaine; L- - - lidocaine; A---A: dibucaine; tetracaine.

Fig. 2. Effects of several local anesthetics on the oxidation of 5-HT and PEA by rat liver mitochondria. MAO at pH 7.4 and 8.4. Conditions and symbols were as for Fig. 1. of 1 x 10⁻³ M. The order of inhibitory potencies was tetracaine > procaine > dibucaine > prilocaine > lidocaine. Tetracaine and dibucaine inhibited PEA oxidation as much as 5-HT oxidation, but procaine inhibited PEA oxidation less than 5-HT oxidation, that is, it inhibited MAO activity only 30% even at 1 x 10⁻³ M. The order of inhibitory potencies was tetracaine > dibucaine > procaine > prilocaine > lidocaine.

Effects of local anesthetics on MAO activity in liver mitochondria: All local anesthetics tested in this experiment at concentrations of 1 x 10⁻⁷ M to 1 x 10⁻³ M inhibited MAO activity in liver mitochondria with 5-HT as substrate. The IC₅₀ values of tetracaine, procaine, dibucaine and lidocaine were 1 x 10⁻⁶ M, 1 x 10⁻⁵ M, 2 x 10⁻⁴ M and 7 x 10⁻⁴ M, respectively. Prilocaine at a concentration of 1 x 10⁻³ M inhibited MAO activity in liver mitochondria about 40%. The local anesthetics inhibited PEA oxidation less than 5-HT oxidation. The IC₅₀ values of tetracaine and dibucaine were 7 x 10⁻⁶ M and 6 x 10⁻⁴ M, respectively. Procaine, lidocaine and prilocaine at concentrations of 1 x 10⁻³ M caused only about 25% inhibition of PEA oxidation (Fig. 1C, D).

Figure 2 shows the effect of pH on MAO inhibition by local anesthetics with 5-HT and PEA as substrates. Tetracaine inhibited MAO activity with 5-HT and PEA as substrates at pH 8.4 than at pH 7.4.

Reversibility of effects of local anesthetics on MAO: The reversibilities of the effects of tetracaine, procaine and dibucaine were tested with 1 x 10⁻⁴ M 5-HT as substrate. The preparations of rat liver mitochondria were mixed with 1 x 10⁻⁴ M tetracaine or procaine, or 1 x 10⁻³ M dibucaine or with only 1 x 10⁻² M Tris-HCl buffer without local
anesthetics as the control. The mixtures were dialyzed overnight against $1 \times 10^{-3}$ M Tris-HCl buffer (pH 8.0) at 10°C, and then their activities were compared. Inhibition of activity by all these drugs except dibucaine was reversed during dialysis. Therefore, it is concluded that the inhibition of MAO activity by dibucaine is irreversible, but that the inhibitions by other drugs are reversible.

Modes of inhibition of local anesthetics: The modes of inhibition by tetracaine, procaine and dibucaine were determined from Lineweaver-Burk double reciprocal plots (Fig. 3). The $K_m$ values for 5-HT of rat liver mitochondrial MAO was $1.5 \times 10^{-4}$ M. The four lines for both tetracaine and procaine intersected at the same point on the ordinate, indicating that the inhibitions by tetracaine and procaine are competitive. However, the four lines for dibucaine intersected at the same point on the abscissa, indicating that its inhibition is non-competitive.

![Fig. 3. Lineweaver-Burk plots for inhibition of 5-HT oxidation by procaine (A), tetracaine (B) and dibucaine (C). Various concentrations of 5-HT were incubated in the presence and absence of drugs and rat liver mitochondrial MAO. Experiments were carried out at pH 7.4.](image)

![Fig. 4. Effects of several local anesthetics on hypotonic hemolysis of rat erythrocytes. Cells were incubated for 1 hr in the dark at 37°C in phosphate buffer corresponding to 0.4% saline in osmolarity. Hemolysis in the control (without any drug) was 70% of the total. Experiments were carried out in duplicate.](image)
Effects of local anesthetics on rat erythrocyte membranes: Figure 4 shows the membrane stabilizing and lytic effects of local anesthetics on erythrocytes. Dibucaine inhibited hypotonic hemolysis at $1 \times 10^{-5}$ M to $4 \times 10^{-4}$ M, but enhanced the hemolysis at $1 \times 10^{-3}$ M. Tetracaine also had a protective effect at $1 \times 10^{-4}$ M to $1 \times 10^{-3}$ M and an enhancing effect at above $2 \times 10^{-3}$ M. Procaine, lidocaine and prilocaine did not inhibit hypotonic hemolysis at up to $1 \times 10^{-2}$ M.

Surface anesthetic effects of local anesthetics on rabbit cornea: Figure 5 shows the surface anesthetic effects of local anesthetics on cornea. Dibucaine and tetracaine completely anesthetized rabbit cornea at $8 \times 10^{-5}$ M and $8 \times 10^{-4}$ M, respectively. The order of potencies of local anesthetics was dibucaine > tetracaine > prilocaine > lidocaine > procaine.

DISCUSSION

The existence of two different functional forms of MAO termed type A and type B MAO has been widely accepted (7, 8). Type A MAO preferentially oxidizes 5-HT and norepinephrine, while type B MAO preferentially oxidizes PEA and benzylamine.

Our results show that the anesthetics tested in this study inhibited brain and liver MAO in different manners, with $I_{50}$ values for inhibition of 5-HT oxidation lower than the concentrations required for surface anesthesia. All the local anesthetics used were tertiary amines except prilocaine (secondary amine) and so they could act as substrate analogues. Tetracaine and procaine inhibited 5-HT oxidation much more than PEA oxidation. At very low concentrations, both were selective type A MAO inhibitors. Kinetic and dialysis studies showed that the inhibitions of 5-HT oxidation by tetracaine and procaine were both competitive and reversible. Dibucaine and prilocaine inhibited PEA oxidation as much as 5-HT oxidation at high concentrations, the inhibition of 5-HT oxidation by dibucaine being noncompetitive.

Fowler et al. (15) reported the effects of various lipophilic compounds including local anesthetics on MAO activity and showed that alcohols were selective MAO-B inhibitors while local anesthetics (tertiary amine type) were selective MAO-A inhibitors.

Wada and Yasuhara reported that propranolol and other $\beta$-blockers inhibited MAO activity in brain and liver and suggested that this effect may be related to the local
anesthetic property of these drugs (16). Thus, membrane stabilizing effects seem to be important in inhibition of MAO activity. Recently Huang reported that MAO was sensitive to the physical state of bulk membrane lipids through lipid-protein interactions, that the active site of the MAO-A enzyme was buried in the hydrocarbon core, and that the functional state was intimately modulated by the fluidity of the hydrophobic region proximal to the polar surface (9).

Then we examined the membrane effects of local anesthetics in preventing hemolysis of erythrocytes and their surface anesthetic action on rabbit cornea. Tetracaine and procaine did not have membrane stabilizing actions at the concentrations required for inhibition of 5-HT oxidation. These results suggest that membrane stabilizing actions of tetracaine and procaine are not important for their inhibition of 5-HT oxidation. On the other hand, the concentrations of dibucaine, lidocaine and prilocaine required for inhibition of 5-HT oxidation were similar to those for membrane stabilization. Lower concentrations of dibucaine had a membrane stabilizing action, whereas a concentration of 1×10⁻³ M caused lysis. Huang and Faulkner (17) reported the effects of several phospholipases on MAO activity and showed that treatment of the enzyme with phospholipase A hydrolyzed almost all phospholipids and disintegrated the outer mitochondrial membrane with concomitant loss of both MAO-A and MAO-B type activities. Our results suggest that dibucaine at high concentration may destroy the phospholipid-protein interaction that is essential for MAO activity.

Local anesthetics inhibit several membrane-bound enzymes (18–22). A preparation of cytochrome oxidase was found to contain about 20% phospholipid, and this phospholipid was apparently essential for oxidase activity because delipidated enzyme was inactive (18). This is also the case with MAO. Dibucaine inhibited cytochrome oxidase with a Ki value of 2 mM. The order of inhibitory potency of the local anesthetics was dibucaine > tetracaine > procaine. This sequence was identical with their relative orders of membrane stabilizing action in our experiment. Cationic local anesthetics have been suggested to cause mixed type inhibition of cytochrome oxidase that appears to depend on both electrostatic and non-polar interactions of the anesthetics with the enzyme. If the inhibitions of MAO by anesthetics can be explained in the same way, it can be supposed that the charged portions of tetracaine and procaine probably compete with the substrate 5-HT for binding because type A MAO inhibition by tetracaine was reduced with increase in pH; while in the case of dibucaine, hydrophobic interactions between the anesthetic and oxidase-associated phospholipids results in formation of a non-productive complex. Other local anesthetics may act on MAO by both mechanisms. Tetracaine is more lipophilic than procaine judging from its partition coefficient in n-heptane-buffer (23). Lipophilicity seems to be related to proximity to the region of the active center of MAO. This may explain the difference between the inhibitions of MAO by tetracaine and procaine. Houslay suggested that the selective actions of clorgyline and deprenyl result from their local concentrations in the region of the active centers of the enzyme forms due to lipid partition effects (24). Huang and Faulkner (17) suggested that the active site of MAO-B may be situated close to or inside the peripheral hydrophilic region and that its functional state may be largely dependent upon the ionic and polar characteristics of the surface polar head layer of the membrane. But our results show that inhibition of PEA oxidation was only caused by drugs having a potent membrane stabilizing
action which prevents hemolysis and potent surface anesthetic activity or having a high oil/water partition coefficient. Thus there is some disagreement over the interpretation of the functional state of type B MAO.

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