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Effect of Ether, Chloroform and Carbon Dioxide on Monoamine Inactivation

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FELDMAN, J. AND J. ROCHE. *Effect of ether, chloroform and carbon dioxide on monoamine inactivation.* PHARMAC. BIOCHEM. BEHAV. 4(4) 447–453, 1976. – To determine if anaesthetic agents alter monoamine inactivation, we exposed tissue homogenates (liver, kidney and brain) from mice and rabbits to ether and chloroform vapors and carbon dioxide gas. These anaesthetic agents inhibited monoamine oxidase (MAO) activity against tryptamine and serotonin. Concentrations of anaesthetic agents that are achieved in the plasma of man during general anaesthesia caused a 27% (ether) and 49% (chloroform) reduction in mouse liver MAO; higher concentrations caused a 95% inhibition mouse or rabbit liver MAO. Kinetic analysis with tryptamine as substrate indicate that ether and chloroform are noncompetitive, reversible MAO inhibitors that preferentially inhibit Type B MAO. Ether and chloroform cause noncompetitive inhibition of serotonin oxidation by mouse liver MAO and competitive inhibition of serotonin oxidation by mouse brain and kidney MAO. Ether or chloroform did not alter catechol-O-methyltransferase activity from tissues of mice. Isolated blood platelets (rabbit and human) were used as a model system for neuronal uptake. Ether caused an irreversible inhibition of serotonin uptake by platelets.

Monoamine Oxidase Diethylether Chloroform Carbon dioxide Enzyme inhibitors
Catechol-O-methyltransferase General anaesthesia Serotonin Catecholamines

PLASMA catecholamine concentration is increased during diethylether (ether)-induced general anaesthesia. In man the elevation in plasma catecholamine concentration is predominantly due to norepinephrine (NE) [25] while in the dog and cat it is due to epinephrine (E) [24]. Some reports indicate the concentration of serotonin in rat tissues is elevated by ether anaesthesia [1,4], while other reports indicate that the tissue concentration of serotonin (5-HT) is not altered by this drug [11,23]. The increased monoamine concentrations during ether anaesthesia has been attributed to increased secretion (NE, E.) or increased synthesis (5-HT) of monoamines. Such results might also occur if ether decreased the inactivation of monoamines.

Catechol and indolamines are inactivated by the enzyme monoamine oxidase (monoamine: oxygen oxidoreductase, 1.4.3.4, MAO) and a neuronal and extraneuronal transmitter uptake mechanism [16,26]. Catecholamines are also inactivated by the enzyme catechol-O-methyltransferase (s-adenosylmethionine: catechol-O-methyl-transferase, 2.1.1.6, COMT) [26]. In the present study we have determined if high concentrations of ether can inhibit these mechanisms. We also compared the effect of ether on MAO with the effect of other general anaesthetics (chloroform and carbon dioxide [CO_2]) and anoxia on this enzyme. Because of the species and organ difference in the susceptibility of MAO isoenzymes to inhibition by MAO inhibitors we evaluated the effect of these gases on liver, kidney and brain MAO from rabbits and mice [28,31]. We used blood platelets as a model system for the evaluation of ether on neuronal uptake. Platelets, which share many properties with the presynaptic nerve terminals, were used as a model system for the evaluation of neuronal uptake [27].

METHOD

In Vitro Studies

Male albino rabbits (weight 1.0 to 1.5 Kg) and male Swiss-Webster albino mice (weight 18–24 g) were sacrificed by cervical dislocation. Samples of liver, kidney cortex and occipital cerebral cortex were obtained from rabbits; samples of liver, kidney cortex and the entire brain were obtained from mice. After the tissues were homogenized in 1.1% potassium chloride, an aliquot of each tissue was taken for a protein determination while additional aliquots were diluted with the appropriate phosphate buffer in preparation for the enzyme assays. The assay tubes contained the following final volumes: tryptamine MAO assay 375 μl; serotonin MAO assay 1 ml; and COMT assay 150 μl.

The tubes were then placed in 5 glass beakers on a reciprocating shaker. Nothing was added to the first beaker containing the control tubes. The paper on the bottom of the second beaker was saturated with ether, paper on the bottom of the third beaker was saturated with chloroform, and a chip of dry ice (CO_2) was placed on the bottom of the fourth beaker. The beakers were then immediately tightly sealed. A stream of 100% nitrogen and a mixture of 95% oxygen–5% carbon dioxide (95% O_2–5% CO_2) was directed into partially sealed fifth and sixth beakers. After 15 min exposure to the gas (ambient temp of 21 to 24°C),

the beakers were opened and the tubes removed. The ^{14}C labeled substrate was added (tryptamine and serotonin in 25 μl, s-adenosylmethionine in 100 μl), the tubes were placed in a gyrotary water bath (ambient air) and the enzyme activity was determined. When studies were done to determine if the effects of the anaesthetic agents were reversible, the tubes were exposed to ether, chloroform and CO_2 with appropriate controls, and then left open at room temperature for 2 to 10 hr. The tubes evaluated at 24 hr were kept uncovered in the refrigerator at 4°C.

In the studies to determine if the concentration of ether and chloroform found in the plasma during general anaesthesia can alter MAO activity, appropriate amounts of ether or chloroform in phosphate buffer were added directly to the tubes, which were then sealed with a glass stopper for the enzyme assay. This technique was also used during the kinetic studies to determine the characteristics of MAO inhibition by ether and chloroform.

For the preparation of isolated platelets, blood was collected (into siliconized tubes containing sodium citrate) from the central ear artery of rabbits and the antecubital vein of human volunteers. Platelet rich plasma was obtained by centrifuging the blood for 15 min at 95 x G in a refrigerated centrifuge. To isolate platelets for MAO assay the platelet–rich plasma was centrifuged for 15 min at 1,100 x G in the refrigerated centrifuge. After 1 wash with 0.9% sodium chloride, the platelets were homogenized in 1.1% potassium chloride. Aliquots of homogenate were assayed for MAO activity and for protein content. The MAO activity was expressed as pmole product formed/mg platelet protein/min.

To evaluate the effect of ether on 5-HT uptake by platelets, tubes containing 0.75 ml of platelet-rich plasma were exposed to room air or ether vapors (21 to 24°C) for 15 min as previously described. Ten μl of ^{14}C serotonin was then added to each tube. The serotonin that was added would increase the plasma serotonin concentration to 1.3×10^{-5} M. The 0 time specimen was immediately cooled to 0°C, and spun in a refrigerated centrifuge at 1,100 x G for 15 min. The remaining tubes were incubated in a gyrotary water bath at 37°C for 30 to 120 min and then centrifuged. After being washed with 3 ml of 0.9% sodium chloride twice, the platelets were transferred to a vial containing 10 ml of Bray's Scintillation fluid [3], sonicated for 1 min with a Sonifier Cell Disrupter (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) and counted in a liquid scintillation counter. The platelet serotonin uptake was expressed as nmoles serotonin/10^{11} platelets.

In Vivo Studies

After obtaining a blood sample from their central ear artery for platelet isolation, rabbits were anaesthetized with open drop ether. After being maintained in the third stage of anaesthesia for 15 min, a second arterial blood sample was obtained.

Mice were randomly divided into 2 groups. One group was immediately sacrificed and a piece of liver removed for MAO assay. The second group was anaesthetized with ether for 15 min (third stage anaesthesia) before a piece of liver was removed for MAO assay.

Analytical Procedures

MAO activity was determined using tryptamine, 3.5×10^{-7} M [30] or serotonin, 4.0×10^{-6} M [22] as substrates. COMT was determined by the method of McCaman [15] and protein by the method of Lowry [20]. Plasma platelet counts were determined with a Thrombo counter (Coulter Electronics Inc., Hialeah, Fla.). The concentration of ether and chloroform in the tissue homogenates after exposure to the anaesthetic vapors was determined by gas-liquid chromatography (GLC) [19]. We used a GC45 GLC system equipped with a 25 cm linear recorder and a disc integrator (Beckman Co., Palo Alto, California). The carbon dioxide and oxygen tension in the homogenates was measured with a 313 pH/Blood Gas Analyzer (Instrumentation Laboratory, Lexington, Ma.).

Chemicals

The following isotopes and chemicals were purchased: tryptamine bisuccinate (side chain-2-^{14}C, specific activity 60 mCi/mmole) (New England Nuclear, Boston, Mass.); serotonin creatinine sulfate (side chain-2-^{14}C, specific activity 55 mCi/mmole) and S-adenosyl-L-methionine methyl-^{14}C, specific activity 57 mCi/mmole) (Amersham, Searle, Arlington Heights, Ill.); and 3,4-dihydroxybenzoic acid, tryptamine HCl and serotonin creatinine sulfate (Cal Biochem La Jolla, California).

Statistical Analysis

When multiple experimental groups are compared with a control group, the data is examined by analysis of variance and by Dunnett's single tailed multiple comparison test. When a single experimental group is compared with a control group, the Student's t test is used [32]. The characteristics of MAO inhibition by ether and chloroform were analyzed by the double-reciprocal plot method of Lineweaver-Burk [12].

RESULTS

MAO

Table 1 depicts the effect of exposure to various gases and vapors on the MAO activity of homogenates of liver, kidney and brain tissue obtained from mice and rabbits, using tryptamine as a substrate. Ether, chloroform and CO_2 significantly decreased the MAO activity of these tissues in both species. Anoxia does not account for these results for 100% nitrogen does not decrease MAO activity. A non-anaesthetic elevation of CO_2 concentration (95% O_2 –5% CO_2) does not alter MAO activity. In additional studies using tryptamine as the substrate, ether produced an 88 to 94% inhibition of MAO obtained from human and rabbit platelets. Ether and chloroform also inhibited liver, kidney and brain MAO when serotonin was used as the substrate (Table 2). The concentration of ether in the homogenates of mouse tissue as determined by GLC was: liver, 135 mM, kidney 270 mM and brain 350 mM. The concentration of chloroform in the mouse tissue homogenates as determined by GLC was: liver 11.0 mM, kidney 16.5 mM and brain 18 mM. These concentrations are 7 to 27 greater than the mean plasma ether (13 mM) and chloroform (1.6 mM) concentrations in man during anaesthesia with these agents [8,15]. After exposure to the CO_2 gas released from dry ice the partial pressure of CO_2 in the mouse tissue homogenates ranged from 150 to 180 mm Hg (mean 160 mm Hg) and the partial pressure of oxygen in the homogenates ranged from 102 to 118 mm Hg (mean 110 mm Hg). After exposure to 95% O_2 –5% CO^2 the partial

TABLE 1

THE EFFECT OF ANAESTHETIC VAPORS AND GASES ON MAO ACTIVITY OF TISSUE HOMOGENATES OF MICE AND RABBITS WITH TRYPTAMINE SUBSTRATE§

Tissue	MAO Specific Activity (pmoles/mg/min) Control	Ether	Chloroform	CO_2	Nitrogen	CO_2 (95%) O_2 (5%)
Mice						
Liver	10.7±1.11	0.6±0.21*	0.90±0.27*	4.4±0.67*	8.5±1.16	8.7±1.51
Kidney	11.3±2.52	2.1±0.37*	1.9±0.50*	4.7±0.93*	10.5±1.92	10.1±2.22
Brain	25.2±2.44	8.7±1.47*	6.7±0.67*	19.5±2.35*	24.9±2.32	25.7±2.61
Rabbit						
Liver	19.9±2.00	2.0±0.45*	2.2±0.75*	9.6±1.76*	16.7±1.15	16.7±1.34
Kidney	15.4±1.56	1.7±0.16*	2.5±0.61*	8.8±1.20*	16.0±1.50	15.1±1.98
Brain	14.5±1.85	2.2±0.16*	2.7±0.57*	9.5±1.35†	16.0±2.30	15.3±2.00

§Each value represents: mice M±SEM of 8 observations; rabbits M±SEM of 6 observations.
*Significantly lower than control MAO of tissue at $p<0.01$ (Dunnett's Single Tail Test).
†Significantly lower than control MAO of tissue at $p<0.05$ (Dunnett's Single Tail Test).

TABLE 2

THE EFFECT OF ANAESTHETIC VAPORS AND GASES ON MAO ACTIVITY OF TISSUE HOMOGENATES OF A MOUSE AND RABBIT WITH SEROTONIN SUBSTRATE

Tissue	MAO Specific Activity (p moles/mg/min) Control	Ether	Chloroform	CO_2
Mice				
Liver	40.2	7.8	4.8	29.7
Kidney	68.2	29.7	8.8	39.1
Brain	182.9	65.1	35.9	94.3
Rabbit				
Liver	42.9	3.1	6.2	14.8
Kidney	76.5	21.1	13.1	48.2
Brain	69.2	14.8	7.2	28.3

pressure of CO_2 in the homogenates ranged from 10–14 mm Hg (mean 12 mm Hg).

Ether or chloroform were added to a homogenate of mouse liver to achieve a concentration of these anaesthetics in the assay tube, that is present in the blood and the liver during general anaesthesia. There were 6 tubes of homogenate in each experimental group. The MAO activity of the control homogenate was 28 ± 1.4 p mole/mg/min. There was a 27% reduction in MAO activity in the presence of 13 mM ether (20 ± 0.7 p moles/mg/min); there was a 49% reduction in MAO activity in the presence of 1.6 mM chloroform (15 ± 0.5 p moles/mg/min). Analysis of variance and Dunnett's test indicated that both ether and chloroform significantly inhibited hepatic MAO.

To determine the characteristics of MAO inhibition by ether and chloroform kinetic studies were done with varying tryptamine concentrations. Figure 1 shows a double-reciprocal plot of a representative study with homogenates of mouse liver and brain. Ether decreases the maximum velocity (V_{max}) but does not alter the Michaelis constant (K_m) of liver and brain MAO, indicating that ether is a non-competitive MAO inhibitor. In additional studies not depicted, ether was found to be a non-competitive inhibitor of kidney MAO, and chloroform was found to be a noncompetive inhibitor of liver, brain and kidney MAO.

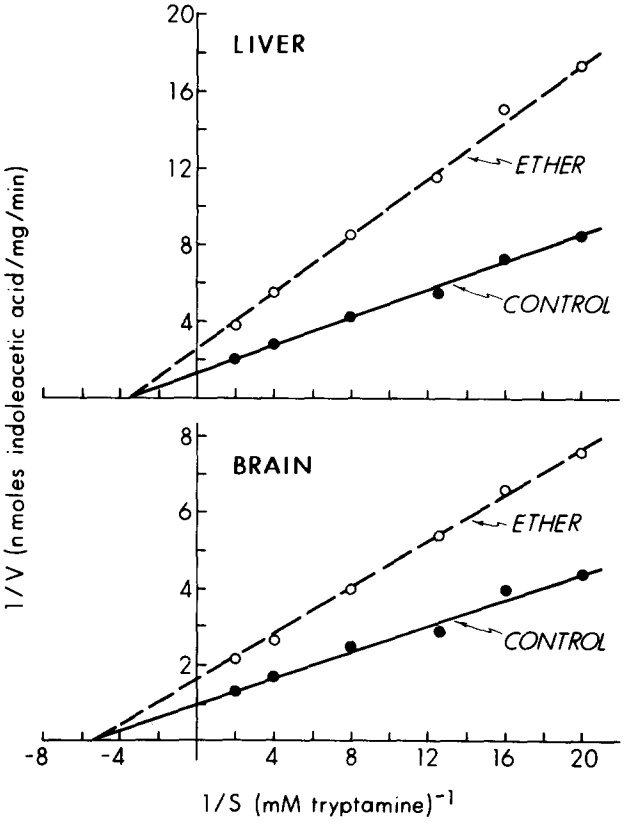

FIG. 1. Double reciprocal plot of the concentration of tryptamine in the assay mixture versus the velocity of the MAO reaction in the absence (CONTROL) and in the presence of ether. Ether produced noncompetitive inhibition of MAO from mouse liver and brain.

Table 3 compares the K_m, the V_{max} and the dissociation constant of the enzyme-inhibitor complex for ether and chloroform (K_i) with the percent of Type A and Type B MAO found in the tissue. The K_i is also the

TABLE 3

RELATIONSHIP BETWEEN THE TYPE OF MAO IN MOUSE TISSUE, THE Km AND Vmax FOR TRYPTAMINE AND SEROTONIN AND THE INHIBITION OF MAO BY ETHER AND CHLOROFORM

	Percent Total MAO		Tryptamine				Serotonin			
			Km	Vmax	Ki (mM)		Km	Vmax	Ki (mM)	
	Type A	Type B	(μM)	(p mole/mg/min)	Ether	Chloroform	(μM)	(p mole/mg/min)	Ether	Chloroform
Liver	4%	96%	29	714	34	2.0	570	400	56	1.4
Brain	40%	60%	19	1000	203	13.4	250	1100	61*	6.6*
Kidney	66%	34%	17	416	276	7.4	166	475	46*	2.1*

*Competitive inhibition. Remainder of Ki values were calculated on the basis of noncompetitive inhibition.

inhibitor concentration required to slow the MAO reaction to half the rate that occurs in the absence of inhibitor. Liver MAO is predominantly of the B Type and the K_i of ether is lowest for liver MAO. There is less Type B MAO in brain and kidney and the K_i of ether for MAO from these tissues is higher. The K_i for inhibition of MAO by chloroform is lowest for liver MAO, intermediate for kidney MAO and highest for brain MAO.

Figure 2 depicts the effect of ether on MAO activity of homogenates of mouse liver and brain using serotonin as a substrate. The serotonin concentrations used were higher than the tryptamine concentrations as serotonin has a larger K_m for MAO than does tryptamine. Ether again was a noncompetitive inhibitor of liver MAO. However, it was a competitive inhibitor of brain MAO (no effect on V_{max} but an increase in K_m). In additional studies not depicted, ether was found to completely inhibit serotonin oxidation by kidney MAO. Chloroform noncompetitively inhibited serotonin oxidation by liver MAO and competitively inhibited serotonin oxidation by brain and kidney MAO. Table 3 shows that liver MAO has the highest K_m for serotonin while kidney MAO has the lowest K_m for serotonin reflecting the greater content of Type B MAO in kidney. The K_i for inhibition of liver MAO by ether and chloroform with serotonin as substrate was similar to that noted using tryptamine as a substrate. The K_i for inhibition of brain and kidney MAO with serotonin as substrate was lower than that noted using tryptamine as a substrate.

Studies were done to determine if the inhibition of MAO by ether using tryptamine as a substrate, could be overcome with high concentrations of tryptamine. Table 4 shows that tryptamine concentrations that greatly exceed K_m can partially overcome MAO inhibition by ether. Similar results were noted when brain and kidney MAO were assayed with high concentrations of tryptamine.

Figure 3 shows a study designed to determine if inhibition of mouse liver MAO by ether is reversible or if it is irreversible. Immediately after exposure to ether, MAO activity is 12% of control. Within 2 hr the MAO activity is 53% of control. Following a plateau period of 6 hr the MAO activity again increases so that by 24 hr it has returned to 89% of control. Studies were also done to determine if MAO inhibition by chloroform and CO_2 is reversible. Immediately after exposure to chloroform the MAO activity of mouse liver is 3 ± 0.2% of control. Two hr after exposure it is 8 ± 1.02% of control and by 24 hr it is 89 ± 8.1% of control. Immediately after exposure to CO_2 the MAO activity is 39 ± 1.2% of control. By 2 hr it has returned to 107 ± 4.1% of control.

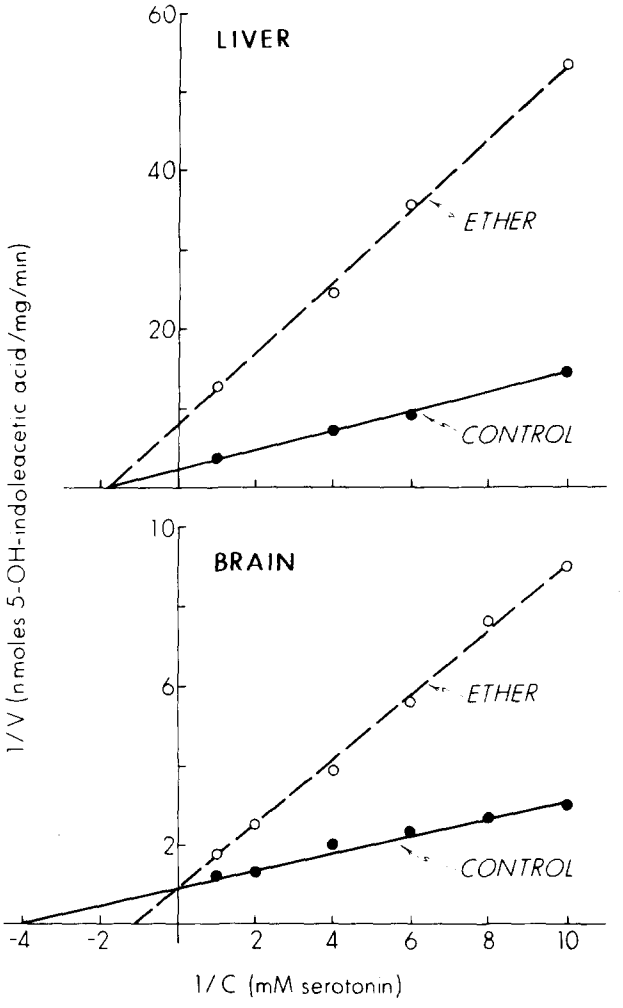

FIG. 2. Double reciprocal plot of the concentration of serotonin in the assay mixture versus the velocity of the MAO reaction in the absence (CONTROL) and in the presence of ether. Ether produced noncompetitive inhibition of MAO from mouse liver and competitive inhibition of MAO from mouse brain.

COMT

Table 5 shows that the COMT activity of mouse liver, kidney and brain is not altered by exposure to ether or chloroform.

TABLE 4

EFFECT OF HIGH CONCENTRATIONS OF TRYPTAMINE ON INHIBITION OF LIVER MAO BY ETHER AND CHLOROFORM

Tryptamine (μM)	MAO Percent of Control Ether (33 mM)	Chloroform (2 mM)
5.0	52	53
6.3	49	53
8.0	48	50
12.5	50	46
25.0	52	46
50.0	52	49
100.0	56	52
250.0	74	78
1000.0	86	84

The velocity of the MAO reaction of the control groups ranged from 52 p moles/mg/min with 5 μM tryptamine to 567 p moles/mg/min with 1000 μM tryptamine.

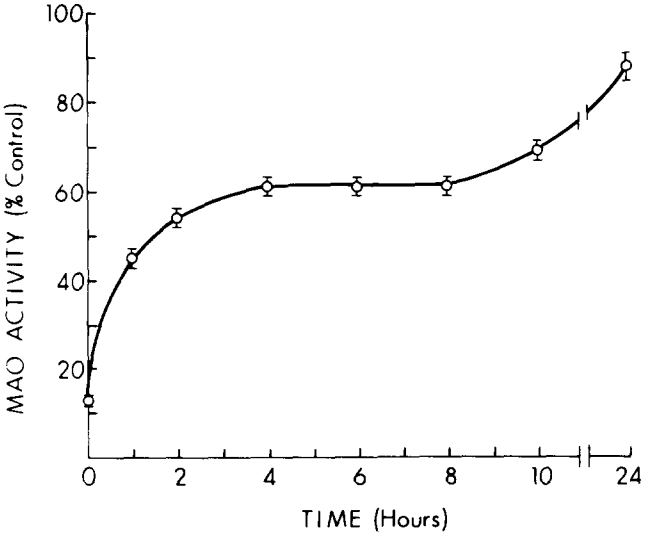

FIG. 3. Time course of recovery of mouse liver MAO activity tryptamine substrate after exposure to ether. The MAO specific activity of the control tissues at 0 time was 17 p moles/mg/min. Each point represents the mean and the brackets ± 1 SEM of 5 experimental observations.

Serotonin Uptake By Platelets

Figure 4 shows that platelets from normal volunteers have a rapid uptake of ^{14}C-serotonin. Prior exposure of the platelets to ether vapor causes more than 90% inhibition of serotonin uptake. To determine if this effect is reversible platelets were allowed to stand for 2 and 24 hr after exposure to ether. There was no recovery of the ability to take up ^{14}C labeled serotonin even 24 hr after being removed from the ether.

The platelet MAO activity of 7 rabbits was measured prior to and after 15 min of general anaesthesia. The MAO activity of the platelets after exposure was 4.1 ± 0.47 p moles/mg/min. The MAO activity of the platelets after exposure was 4.1 ± 0.72 p moles/mg/min. In the 2 rabbits in which it was evaluated, exposure to ether did not alter the ^{14}C serotonin uptake of the platelets. There was no significant difference between the liver MAO activity of 5 control mice (29 ± 2.8 p moles/mg/min) and the liver MAO activity of 5 mice that had been anaesthetized with ether for 15 min (33 ± 5.4 p moles/mg/min).

TABLE 5

THE EFFECT OF ETHER AND CHLOROFORM ON COMT ACTIVITY IN MOUSE TISSUES

Tissue	COMT (p moles product/mg/min) Control	Ether	Chloroform
Liver	103±9.8§	101±9.1	97±10.7
Kidney	43±7.5	47±5.1	44±7.7
Brain	9±0.7	10±0.7	10±0.5

§Each value represents Mean ± SEM of 10 observations.

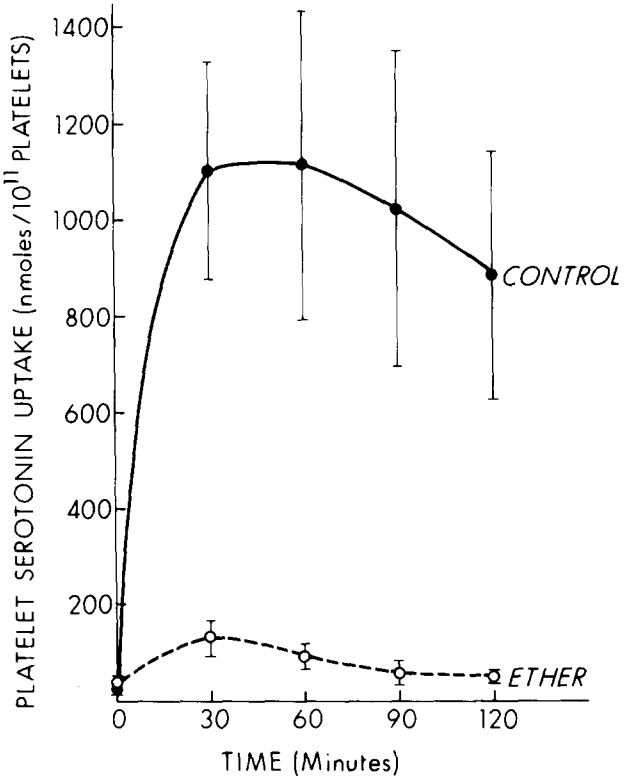

FIG. 4. Effect of ether on the serotonin uptake of platelets obtained from normal human subjects. Each point represents the mean and the brackets ± 1 SEM of observations made on the platelets of 6 subjects. Ether exposure significantly reduced the serotonin uptake ($p<0.05$) at all time points.

DISCUSSION

In the present report we noted that ether, chloroform or CO_2 inhibit MAO. In high concentration (30–40%) CO_2 is a general anaesthetic while in lower concentrations (<20%) it is not an effective anaesthetic [18]. Five percent CO_2 did not inhibit MAO. The decrease in MAO activity was not

due to anoxia for there was no loss of MAO activity after tissue homogenates were incubated in 100% N for 15 min. High concentrations of ether also markedly reduce the ability of platelets to take up serotonin. In contrast, ether and chloroform did not alter the COMT activity.

The results of *in vitro* and *in vivo* experiments with rats and mice suggest that there are at least 2 types of MAO [13, 28, 31]. Enzyme A is heat stable, is sensitive to inhibition by clorgyline and harmine and preferentially metabolizes dopamine, serotonin and norepinephrine. Enzyme B is heat labile, is sensitive to inhibition by deprenyl and pargyline and preferentially metabolizes benzylamine and β-phenethylamine. Tryptamine, tyramine and kynuramine are metabolized by both types of MAO.

Ether and chloroform are noncompetitive inhibitors of MAO when tryptamine is used as a substrate. The K_i for ether and chloroform inhibition of tryptamine metabolism by mouse liver MAO is much lower than the K_i values for MAO from brain and kidney. Mouse liver contains predominantly Type B MAO while the other tissues contain a mixture of Type A and Type B suggesting that ether and chloroform preferentially inhibit Type B MAO. Extremely high tryptamine concentrations partially overcome MAO inhibition by anaesthetic agents.

It is somewhat puzzling that ether and chloroform have a lower K_i for inhibiting serotonin metabolism by MAO since serotonin is predominantly metabolized by Type A MAO. The K_m of MAO for serotonin is much greater than that for tryptamine and it may thus be easier to inhibit the oxidation of serotonin by MAO. Ether and chloroform are competitive antagonists of serotonin metabolism by brain and kidney MAO. This is analogous to the findings that the reversible MAO inhibitors phenoxyisopropylamine and 2-chorophenoxyisopropylamine are noncompetitive inhibitors of phenethylamine oxidation and competitive inhibitors of tryptamine oxidation [13]. Oxidation of serotonin by liver MAO is noncompetitively inhibited by ether and chloroform. Liver MAO has a very high K_m for serotonin (Table 3). Serotonin oxidation by liver MAO may be due to Type B MAO, for at high serotonin concentrations, this MAO isoenzyme can oxidize serotonin [28].

An earlier study evaluated the effect of ether on the uptake and metabolism of l-norepinephrine (NE) by contracting guinea pig atria [6]. Ether concentrations that produced a 50% decrease in peak muscle tension did not alter the rate of uptake of NE. The percentage of deaminated NE in the atrium was not altered by ether, suggesting that MAO activity was not altered by this anaesthetic. The results of the present study do not conflict with this study for we used different species (mice and rabbits), different monoamines (tryptamine and serotonin), different tissues (liver, kidney, brain and platelets) and in most experiments a considerably greater concentration of ether.

When rats inhale ether in a concentration that will abolish the righting reflex (3%), there was no change in the brain serotonin concentration [11]. Ether increased the brain serotonin turnover when measured by inhibiting MAO with pargyline or inhibiting 5-HIAA flux out of the brain with probenicid. The authors speculated that ether may increase the rate of hydroxylation of tryptophan, the rate limiting step in serotonin synthesis. From their experimental design they could not determine if ether increased brain MAO activity. Our studies suggest that MAO activity is not increased by ether. We could not demonstrate a decrease in MAO activity in rabbit platelets or mouse liver obtained from animals anaesthetized with ether. When tissues are prepared for MAO assay, 25 mg of tissue is homogenized in 5 to 10 ml of 1.1% KCl. This results in a marked dilution of tissue ether. It also takes a minimum of 15 min to prepare liver and a minimum of 40 min to prepare platelets for MAO assay. In light of the reversible nature of the ether inhibition of MAO (Fig. 3), it is not surprising that we could not demonstrate MAO inhibition in tissues from animals anaesthetized with ether. When ether and chloroform were added to the assay tubes to achieve concentrations found in blood and tissues during general anaesthesia, there was a 24% (ether) and 44% (chloroform) inhibition of MAO [18,19]. This degree of MAO inhibition may not be of physiological significance during general anaesthesia, for most tissues have an excess amount of MAO activity. When rats received conventional MAO inhibitors, brain serotonin increases only after there is a 50 to 85% decrease in MAO [9,17].

Previous studies demonstrate that ether and chloroform have a variety of effects on other enzymes. Chronic exposure of rats to ether increased the activity of hepatic microsomal drug metabolizing enzymes [7] while acute exposure to ether can decrease the activity of these enzymes [2]. Acute exposure of rats to ether or chloroform noncompetitively inhibits liver microsomal glucuronyltransferase activity; ether (but not chloroform) also decreases the formation of the glucuronic acid donor, uridine diphosphoglucuronic acid [5]. Acute exposure to chloroform (but not ether) stimulates hepatic microsomal lipoperoxidation in phenobarbital-pretreated rats [4]. Finally ether can inhibit lactate dehydrogenase [14] and carbonic anhydrase [10]. However, because the effect on lactate dehydrogenase is achieved only at extremely high *in vitro* ether concentrations, and carbonic anhydrase is only inhibited 5.6%, during ether anaesthesia, the effect of ether on these enzymes is probably not of physiological significance.

In the present report we have noted that the volatile anaesthetics ether and chloroform have the novel property of being both noncompetitive and competitive, spontaneously reversible MAO inhibitors. Further studies will be needed to determine if these agents have a clinically significant effect on monoamine oxidase and the neuronal reuptake of monoamines in man during general anaesthesia.

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REFERENCES

1. Anderson, E. G. and D. D. Bonnycastle. A study of the central depressant action of pentobarbital, phenobarbital and diethyl ether in relationship to increases in brain 5-hydroxytryptamine. *J. Pharmac. exp. Ther.* **130:** 138–143, 1960.

2. Baekeland, F. and N. M. Greene. Effect of diethy ether on tissue distribution and metabolism of pentobarbital in rats. *Anaesthesiology* **19:** 724–732, 1958.

3. Bray, G. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Analyt Biochem.* **1:** 279–285, 1964.
4. Brown, B. R. Hepatic microsomal lipoperoxidation and inhalation anesthetics. *Anesthesiology* **36:** 458–465, 1972.
5. Brown, B. R. Effects of inhalation anesthetics on hepatic glucuronide conjugation. *Anesthesiology* **37:** 483–488, 1972.
6. Brown, B. R., E. N. Tatum and J. R. Crout. The effects of inhalation anesthetics on the uptake and metabolism of *l*-^{3}H-norepinephrine in guinea pig atria. *Anesthesiology* **36:** 263–267, 1972.
7. Brown, B. R. and A. M. Sagalyn. Hepatic microsomal enzyme induction by inhalation anesthetics. *Anesthesiology* **40:** 152–161, 1974.
8. Chenoweth, M. B., D. N. Robertson, D. S. Erley and R. Golhke. Blood and tissue levels of ether, chloroform, halothane and methoxyflurane in dogs. *Anesthesiology* **23:** 101–106, 1962.
9. Chessin, M., B. Dubnick, G. Lesson and C. C. Scott. Biochemical and pharmacological studies of β-phenylethylhydrazine and selected related compounds. *Ann. N. Y. Acad. Sci.* **80:** 597–608, 1959.
10. Christian, G. and N. M. Greene. Blood carbonic anhydrase activity in anesthetized man. *Anesthesiology* **23:** 179–186, 1962.
11. Diaz, P. M., S. H. Ngai and E. Costa. The effects of cyclopropane, halothane and diethyl ether on the cerebral metabolism of serotonin in the rat. *Anesthesiology* **29:** 959–963, 1968.
12. Dixon, M. and E. C. Webb. *Enzymes.* New York: Academic Press Inc., 1964, pp. 313–334.
13. Fuller, R. W. Selective inhibition of monoamine oxidase. In: *Monoamine Oxidase, New Vistas,* edited by E. Costa and P. Greengard. New York: Raven Press, 1972, pp. 339–354.
14. Greene, N. M. and E. L. Spencer. Diethyl ether and lactate dehydrogenase activity *in vitro. Anesthesiology* **24:** 23–28, 1963.
15. Halsey, M. J., E. B. Smith and T. E. Wood. Effects of general anesthetics on Na^+ transport in human red cells. *Nature* **225:** 1151–1152, 1970.
16. Iversen, L. L. Role of transmitter uptake mechanisms in synaptic neuro-transmission. *Br. J. Pharmac.* **41:** 571–591, 1971.
17. Gey, K. F. and A. Pletscher. Activity of monoamine oxidase in relation to the 5-hydroxytryptamine and norepinephrine content of rat brain. *J. Neurochem.* **6:** 239–243, 1961.
18. Leake, C. D. The anesthetic properties of carbon dioxide. *J. Pharmac. exp. Ther.* **33:** 280–281, 1928.
19. Lowe, H. J. Flame ionization detection of volatile organic anaesthetics in blood, gases and tissues. *Anesthesiology* **25:** 808–814, 1964.
20. Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193:** 265–275, 1951.
21. McCaman, R. E. Microdetermination of catechol-0-methyltransferase. *Life Sci.* **4:** 2353–2359, 1965.
22. McCaman, R. E., M. W. McCaman, J. W. Hunt and S. M. Smith. Microdetermination of monoamine oxidase and 5-hydroxytryptophan decarboxylase activities in nervous tissues. *J. Neurochem.* **12:** 15–23, 1965.
23. Paasonen, M. K. and N. J. Giarman. Brain levels of 5-hydroxytryptamine after various agents. *Archs int. Pharmacodyn.* **114:** 189–200, 1958.
24. Price, H. L. Circulating adrenaline and noradrenaline during diethyl ether in man. *Clin. Sci.* **16:** 377–383, 1957.
25. Price, H. L., H. W. Linde, R. E. Jones, G. W. Black and M. L. Price. Sympatho-adrenal responses to general anesthesia in man and their relation to hemodynamics. *Anesthesiology* **20:** 563–575, 1959.
26. Sharman, D. F. The catabolism of catecholamines:recent studies. *Br. Med. Bull.* **29:** 110–115, 1971.
27. Sneddon, J. M. Blood platelets as a model for monoamine-containing neurones. *Prog. Neurobiol.* **1:** 151–198, 1973.
28. Squires, R. F. Multiple forms of monoamine oxidase in intact mitochondria as characterized by selective inhibitors and thermal stability:a comparison of eight mammalian species. In: *Monoamine Oxidase – New Vistas,* edited by E. Costa and P. Greengard. New York: Raven Press, 1972, pp. 355–370.
29. Thompson, J. H. and L. B. Campbell. The effect of several anaesthetic agents upon the 5-hydroxytryptamine concentration in the jejunal mucosa of the Sprague-Dawley rat. *Archs int. Pharmacodyn* **169:** 1–5, 1967.
30. Wurtman, R. J. and J. Axelrod. A sensitive and specific assay for the estimation of monoamine oxidase. *Biochem. Pharmac.* **12:** 1439–1441, 1963.
31. Youdim, M. B. H. Multiple forms of mitochondrial monoamine oxidase. *Br. Med. Bull.* **29:** 120–122, 1973.
32. Zar, J. H. Biostatistical Analysis. Englewood Cliffs, N.J.: Prentice Hall, 1974.