Anesthesiology
V 39, No 6, Dec 1973

Effects of Halothane and Nitrous Oxide on Removal of Norepinephrine from the Pulmonary Circulation

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The effects of halothane and nitrous oxide on norepinephrine (NE) removal by isolated perfused rabbit lungs were measured by determining the rates of removal of NE from the pulmonary arterial circulation. Halothane (0.5, 1.0, and 2.0 per cent) decreased uptake by 12.9, 23.9, and 27.6 per cent. Nitrous oxide (50 per cent) reduced NE removal by 24 per cent. Combining 0.5 per cent halothane with 50 per cent nitrous oxide produced additive inhibition of removal. (Key words: Norepinephrine; Lung perfusion; Nitrous oxide; Halothane; Pulmonary vascular space.)

NONRESPIRATORY FUNCTIONS of lung include filtering of emboli and platelets, biosynthesis of surfactant, elimination of volatile substances, and participation in maintenance of water and solute balance.[1] Lungs also have the ability to remove the biogenic amines, norepinephrine (NE) and 5-hydroxytryptamine, from pulmonary blood as it traverses them in several species,[2-3] including man.[4]

The mechanism whereby NE is removed from the pulmonary circulation has been studied in isolated lungs perfused either with blood[5] or with physiologic medium.[6-7] The process is inhibited by a variety of drugs, including ouabain, cocaine and phenoxybenzamine,[6-8] but is unaffected by inhibitors of intrapulmonary amine metabolism by monoamine oxidase, or by catechol-O-methyltransferase. This paper describes experiments designed to determine the effects of inhalation anesthetics on removal of NE by lung.

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Received from the Departments of Anesthesiology and Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510. Accepted for publication July 30, 1973. Supported by Grants from the U. S. Public Health Service (HL-13315 and HL-05942) and the Connecticut Heart Association (#435). Assistance was also provided by Ayerst Laboratories, Inc.

Methods

Independent Perfusion and Inflation of Right and Left Lungs

The preparation and technique employed are similar to those reported previously.[7] Male rabbits (1.7–2.5 kg) were given heparin (500 IU/kg) and were then anesthetized by intravenous injection of allobarbital (30 mg/kg) and urethane (120 mg/kg). The thorax was opened, and both the aorta and the pulmonary artery were divided close to the heart, after which the pulmonary veins and inferior and superior venae cavae were cut and the heart was removed. The trachea was then cut and the lungs transferred to a petri dish containing oxygenated, modified Krebs solution (see below) at 37 C. Left and right bronchi, as well as both pulmonary arteries, were cannulated to permit independent inflation and perfusion of each lung. Cannulated lungs were placed in the vertical position within a humid chamber kept warm by insertion in a constant-temperature water bath maintained at 38 C (fig. 1). A Pyrex glass septum divided the lung chamber into two separate sections, thus allowing independent collection of the effluents which flowed from the cut pulmonary veins of the right and left lungs. The perfusion system consisted of two independent sets of tubing (PE 240) immersed in the same water bath as the lung chamber. Passage through these coils raised the temperature of the perfusion medium, measured at the end of the arterial cannula, from 0 to 36.5 C (fig. 1). Each lung was perfused at a constant flow rate of 10 ml/min by means of a Holter perfusion pump; flow rate was independent of pressure up to 250 torr. Each lung was perfused independently for approximately 20 minutes with modified Krebs solution, which previously had been aerated for 30 minutes with either 100

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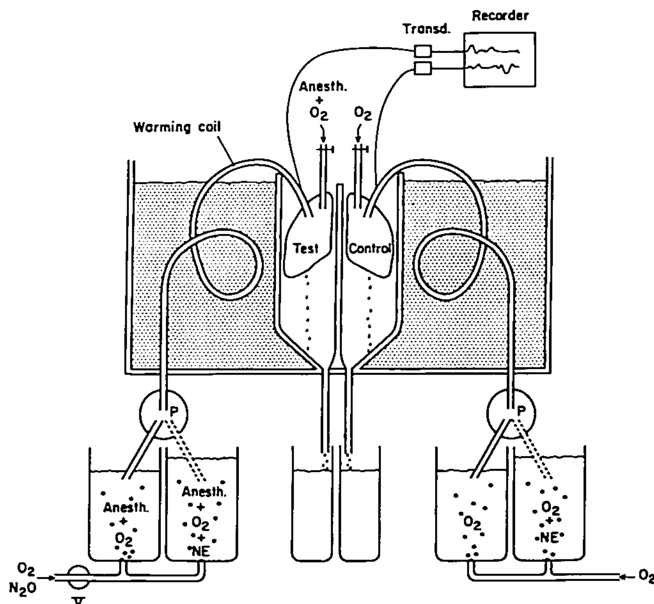

FIG. 1. Schematic diagram of arrangements used for independent perfusion and inflation of lungs with gas mixtures. *V*, vaporizer for halothane; *P*, pump; *NE*, norepinephrine; *Transd*, transducers for pulmonary arterial pressures.

per cent oxygen (control lungs) or anesthetic gas (or a mixture of gases) and oxygen. This period of perfusion is referred to as the "equilibration period." A bubble trap was placed close to the cannula in each pulmonary artery to prevent air emboli.

Control lungs were inflated with 100 per cent oxygen, while test lungs were inflated by means of the anesthetic(s) mixture in oxygen, which also was used for aeration of the perfusion medium. After inflation, a clamp was placed on the bronchial cannula. Left lungs were inflated with 8 ml, right lungs with 12 ml, of gas. Aeration of the perfusion medium with corresponding gases was continued during perfusion.

Halothane was delivered by means of a Fluotec vaporizer, with either 5 l/min of 100 per cent oxygen or 50 per cent oxygen and 50 per cent nitrous oxide as the carrier gas. Nitrous oxide was delivered by a flowmeter (calibrated by the water-replacement method) on the anesthesia machine. Left and right pulmonary arterial pressures were monitored continuously by means of Statham pressure transducers (P23BC), the outputs of which were displayed on a Grass polygraph.

PERFUSION MEDIUM

Krebs medium is normally aerated with a mixture of 95 per cent O_2 and 5 per cent CO_2.[9] Addition of any anesthetic gas, particularly in high concentration (e.g., nitrous oxide), to the premixed 95 per cent oxygen and 5 per cent carbon dioxide mixture signif-

icantly alters carbon dioxide tension in the mixture, thereby changing pH. Therefore, it was necessary to formulate an alternate perfusion solution free of $NaHCO_3$ which maintained a stable pH during aeration with 100 per cent oxygen and which also had good buffering capacity. The pH (at 37 C) of the modified Krebs solution used in this study (table 1) was 7.39–7.41 after 30 minutes of aeration with oxygen or anesthetic(s)/oxygen, and did not change significantly after passing through the lung. A similar modified Krebs solution was used previously to study potential anesthetic effects on uptake of NE by the heart.[10] However, both the ionic concentrations and the osmotic pressure of the solution used in our present study are closer to those of regular Krebs medium (table 1) than those of the previous modification.[10]

TABLE 1. The Compositions of Modified and Normal Krebs Medium

	Modified Krebs Medium		Normal Krebs Medium	
	g/l	mM	g/l	mM
NaCl	7.21	123.4	6.9	118.05
KCl	0.44	5.87	0.35	4.75
$CaCl_2$	0.19	1.71	0.28	2.54
$MgCl_2 \cdot 6H_2O$	0.16	0.79	—	—
Na_2HPO_4	1.3	9.15	—	—
$NaH_2PO_4 \cdot H_2O$	0.22	1.59	—	—
KH_2PO_4	—	—	0.16	1.19
$MgSO_4$	—	—	0.14	1.19
$NaHCO_3$	—	—	2.1	25.0
Glucose	2.0	11.1	2.0	11.1
Total Na^+	3.30	143.3	3.30	143.3
Total K^+	0.23	5.87	0.23	5.87
Total Ca^{++}	0.07	1.71	0.10	2.52
Total Mg^{++}	0.02	0.79	0.03	1.16
Total Cl^-	4.77	134.27	4.54	127.90
mOsm	—	307.77	—	319.08

EXPERIMENTAL DESIGN

After equilibration, both intake tubes were placed in reservoirs containing *dl*-NE hydrochloride in modified Krebs solution, previously aerated with appropriate gases. The perfusion system used has a deadspace of 8 ml; achievement of a steady state of NE concentration within the preparation took 3 minutes. In each of the experiments conducted, perfusion with NE (0.25 μg/ml) was carried out for 10 minutes. In some experiments *d*-NE was perfused through one lung and *l*-NE through the other lung from the same animal. In these instances lungs were inflated with room air.

WATER CONTENT OF LUNGS AFTER PERFUSION

After each experiment the lungs were removed and weighed separately before and after cutting with fine scissors and pressing firmly between filter paper to remove as much fluid as possible. Lung weights used throughout this paper refer to measurements made after such blotting. The difference between wet and blotted weights was used as a measure of water content.

Whenever abnormal pulmonary perfusion occurred (mostly owing to air embolism), a large fluid accumulation was noted on weighing the lungs at the end of the experiment; sometimes massive pulmonary edema was observed to have occurred during the experiment. These experiments were rejected from the study. Water contents of the test and control lungs, as defined above, were identical, and normally were about 20 per cent of the blotted tissue weights.

MEASUREMENT OF AMINE REMOVAL BY LUNG

During amine perfusion, effluents from right and left lungs were collected separately in chilled, actinic glass Erlenmeyer flasks which contained 120 ml 0.2 N perchloric acid. The entire effluent from each lung was collected for a period of 12 minutes (total of 120 ml effluent) beginning 45 seconds after starting NE perfusion. This collection period was used since effluent collected during the period 30–45 seconds after the beginning of NE perfusion contained no measurable amine. Also, no amine could be detected in effluent collected between 2 minutes and 2 minutes, 15 seconds, after stopping NE perfusion (i.e., between 12 minutes and 12 minutes, 15 seconds, after starting perfusion). The concentration of NE in the reservoir containing the perfusion fluid was determined by examining 0.5-ml samples taken at the beginning and end

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TABLE 2. Removal of NE from the Pulmonary Circulation and Its Inhibition by Anesthetics*

Treatment	Norepinephrine Removal (μg/g Lung)		Per Cent Inhibition	Paired t Test†	Student's t Test‡
	Control (100 Per Cent O_2)	Treated (Anesthetic)			
Halothane, 0.5 per cent, in O_2	3.66 ± 0.35	3.16 ± 0.26	12.9 ± 2.8	$P < 0.01$	—
Halothane, 1.0 per cent, in O_2	3.29 ± 0.18	2.48 ± 0.12	23.9 ± 4.7	$P < 0.0025$	$P < 0.05$
Halothane, 2.0 per cent, in O_2	3.49 ± 0.29	2.48 ± 0.21	27.6 ± 6.6	$P < 0.01$	$P < 0.05$
N_2O, 50 per cent, in O_2	3.55 ± 0.30	2.66 ± 0.28	24.0 ± 6.5	$P < 0.0125$	N.S.
Halothane, 0.5 per cent, in 50 per cent N_2O + O_2	2.95 ± 0.22	1.89 ± 0.22	36.7 ± 3.4	$P < 0.0005$	$P < 0.025$

* Means ± SE. Data from six lungs were used to calculate the values presented.
† Significance of mean difference between control and treated lungs.
‡ Significance of per cent inhibition when compared with that produced by 0.5 per cent halothane in O_2.

of every 10-minute perfusion period. The concentration of NE (μg/ml) in 120 ml of effluent, mixed thoroughly with 120 ml of 0.2 N perchloric acid, was determined in duplicate 0.5-ml samples. Norepinephrine was assayed fluorometrically, after ferricyanide oxidation by the method of Udenfriend.[11] In the absence of the lung there was no spontaneous destruction of amine after 10 minutes of NE perfusion through this system. When lungs were perfused, subtraction of the amount of NE in the effluent (NE concentration × 120) from the total amount perfused (NE concentration × 10 ml/min × 10 min) gives the amount of NE removed by the tissue. Total removal of NE, thus calculated, was divided by the blotted weight of lung to yield removal of NE/g of blotted lung. Because of variations in values of removal/g among lungs from different animals, the effects of anesthetics were determined by calculating per cent inhibition using data from control and test lungs taken from the same animal. Per cent inhibition of removal is determined from the formula,

$$\left(1 - \frac{\text{test lung removal/g}}{\text{control lung removal/g}}\right) \times 100.$$

Statistical significance was evaluated by the t test for paired data except in comparisons of percentages of inhibition caused by different anesthetics, for which Student's t test was used.[12]

Results

Pulmonary Arterial Pressure

An increase in pulmonary arterial pressure was noted during NE perfusion. This pressure rise was never more than 3 torr and was the same in both control and test lungs. Changes in pulmonary arterial pressure resulting from anesthetics *per se* were barely detectable.

Removal of *d*- and *l*-NE

Four pairs of lungs were used in this series of experiments. While one lung was perfused with 0.25 μg of *d*-NE/ml, the other was simultaneously perfused with the *l*- isomer at the same concentration. Mean removal of *d*-NE was 2.55 ± 0.32 μg/g, while that of the *l*- isomer was 2.50 ± 0.46 μg/g. These figures are not significantly different; accordingly, NE uptake by rabbit lung does not show stereospecificity.

Effects of Anesthetics

Significant inhibition of NE removal by the lung was observed with each anesthetic at every concentration studied (table 2).

Halothane

There was significant ($P < 0.01$) inhibition of NE removal (12.9 ± 2.8 per cent) with 0.5

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per cent halothane. The inhibition increased significantly ($P < 0.05$) when either 1.0 per cent (23.9 ± 4.7 per cent) or 2.0 per cent (27.6 ± 6.6 per cent) halothane was compared with 0.5 per cent halothane; however, there was no significant difference between the extents of inhibition caused by 1.0 and 2.0 per cent halothane (table 2).

Nitrous Oxide

There was significant ($P < 0.0125$) inhibition of NE removal (24.0 ± 6.5 per cent) with 50 per cent nitrous oxide. However, no significant difference in per cent inhibition of removal was found when the inhibitory effect of nitrous oxide was compared with that of either halothane or a mixture of halothane and nitrous oxide (table 2).

Halothane and Nitrous Oxide Mixture

When 0.5 per cent halothane in 50 per cent nitrous oxide and oxygen was used, there was significant ($P < 0.0005$) inhibition of NE removal (36.7 ± 3.4 per cent). Inhibition was greater than with either 0.5 per cent halothane ($P < 0.025$) or 1.0 per cent halothane ($P < 0.05$) alone. Inhibition caused by the mixture of halothane and nitrous oxide (36.7 ± 3.4 per cent, table 2) seemed to be additive (12.9 per cent inhibition by 0.5 per cent halothane and 24.0 per cent inhibition by 50 per cent nitrous oxide).

Discussion

Although in this study anesthetics were administered to the lung both *via* the pulmonary circulation and by inflating the lung with anesthetics, rather than by continuous ventilation, there was evidence that both the perfusate and the tissue were well equilibrated with anesthetic gas. First, as the perfusion medium, previously aerated with a high concentration of nitrous oxide (80 per cent in oxygen), passed through the warming coils (*i.e.*, reached 36.5 C), bubbles (presumably nitrous oxide) were created in the system; this was particularly evident when the temperature during aeration was below 15 C. Therefore, use of a lower concentration (50 per cent) of nitrous oxide and aeration of warmer solutions was necessary. Second, the halothane concentration in Krebs solution reaches a steady level after only 5 minutes of aeration;[13] we aerated modified Krebs medium for 30 minutes.

There is now evidence indicating that capillary endothelial cells are sites of NE uptake by perfused rabbit lung.[8] Accordingly, if pulmonary capillary perfusion were reduced as a result of precapillary vasoconstriction, NE removal might be lowered in this essentially nonspecific manner. However, the increases of pulmonary arterial pressures in response to NE perfusion were identical in test and control lungs and, furthermore, there was no pressure change due to anesthetic(s) in the perfusion fluid (see Results). Therefore, it is unlikely that there were significant differences in capillary perfusion between control and test lungs which might have explained anesthetic-induced inhibition of NE removal.

When 50 per cent nitrous oxide was added to oxygen, the oxygen tension in the gas and the solution was decreased by half, and thus may have influenced NE removal. However, this is unlikely, since it has been shown in this laboratory that lowered oxygen tension *per se* has no significant effect on removal of NE by perfused rabbit lung.[8]

In the present study, both halothane and nitrous oxide, in the concentrations used, inhibited NE removal by rabbit lung. We reported previously that halothane, methoxyflurane, cyclopropane, and diethyl ether failed to inhibit uptake of tritiated NE by adrenergic nerve endings in slices of cat ventricle.[10] However, uptake into adrenergic nerve terminals shows stereospecificity,[14] while removal of NE from pulmonary vascular space shows no stereospecificity (present results). This fact, together with the above findings, suggests that the mechanisms involved in adrenergic removal and in pulmonary uptake of NE are different. However, the exact mechanism by which anesthetics reduce NE removal by lung is unclear. Certainly, anesthetics are known to depress excitable membranes;[15] perhaps a similar action on the function of endothelial cells (specifically the transport system for NE they are thought to possess) may account for the effects of halothane and nitrous oxide observed in the present study.

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Obstetrics

PRILOCAINE AND PARACERVICAL BLOCK A paracervical block utilizing 16 ml prilocaine was performed in 51 patients in labor. The fetal ECG was monitored. The drug was administered submucosally, rather than deep into the parametria, and no needle guide was used. Eighty-seven blocks were administered to the 51 patients; 92 per cent were considered effective, and the average duration of effectiveness was 55 minutes. Fetal bradycardia, commonly reported to follow paracervical block, was not seen, but early deceleration was noted in one fetus, variable deceleration in 16, and loss of ripple pattern in nine. Five of the fetuses with variable deceleration showed the pattern before the block was given. Six of 51 infants had one-minute Apgar scores below 7. *(Bloom, S. L., Horswill, C. W., and Curet, L. B.: Effects of Paracervical Blocks on the Fetus during Labor: A Prospective Study with the Use of Direct Fetal Monitoring. Am. J. Obstet. Gynecol. 114:218, 1972.)* ABSTRACTER'S COMMENT: It would appear that fetal bradycardia can be avoided if the technique of paracervical block is modified. Although abnormalities in the heart rate appeared, they were probably not of anesthetic origin. Two modifications of the usual paracervical block were introduced (the use of prilocaine rather than mepivacaine or lidocaine and the shallow injection) so that the source of the improved results is not immediately clear. Probably prilocaine is safer than mepivacaine or lidocaine, as it is detoxified more rapidly.

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