Anesthesiology
V 36, No 3, Mar 1972

The Effects of Inhalation Anesthetics on the Uptake and Metabolism of l-^{3}H-Norepinephrine in Guinea-pig Atria

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The uptake and deaminative metabolism of *l*-^{3}H-norepinephrine have been studied *in vitro* in contracting guinea-pig left atria exposed to halothane, cyclopropane, Ethrane, and diethyl ether. In the presence of severe (50 per cent) decreases in myocardial isometric contractile force, no alterations were seen in either the rate of uptake of *l*-norepinephrine or the activity of intraneuronal monoamine oxidase. (Key words: *l*-Norepinephrine; Myocardial contractility; Monoamine oxidase; Cyclopropane; Halothane; Diethyl ether; Ethrane; Catecholamine uptake.)

THE EFFECTS of inhalation anesthetics on the function of postganglionic adrenergic neurons are of considerable interest. Many of these agents alter sympathetic nervous system function in animals and man. Although studies of the uptake of *dl*-norepinephrine in the presence of some of these agents have been carried out,[1,2] the kinetics of uptake of the physiologic *l* isomer may be quite different from those of the *d* isomer.[3] No studies have correlated *l*-norepinephrine uptake with depressed myocardial function during anesthesia, nor have attempts been made to determine the effects of anesthetics on the monoamine oxidase activity of adrenergic neurons. The following studies were conducted with *l*-^{3}H-norepinephrine to determine whether inhalation anesthetics at concentrations which depress myocardial contractility alter either the rate of uptake of the catecholamine transmitter or monoamine oxidase activity within cardiac adrenergic neurons.

Methods

Since the tritiated *l* isomer of norepinephrine was not available commercially at the time these studies were performed, it was synthesized in the laboratory. The *l* isomer of ^{3}H-norepinephrine was prepared by incubating generally labelled ^{3}H-dopamine (New England Nuclear Corp., Boston) with dopamine β-hydroxylase prepared from bovine adrenals by the method of Friedman and Kaufman.[4] After chromatographic purification, the ^{3}H-norepinephrine formed had a specific radioactivity of 5.8 Ci/mmol, migrated like norepinephrine in two solvent systems, had a fluorescence spectrum identical to that of norepinephrine, and was equal in potency to authentic *l*-norepinephrine in the rat blood-pressure assay.

Guinea pigs of either sex weighing 300–400 g were used throughout these studies. Each animal was stunned with a blow to the head and the left atrium was rapidly excised. Under oxygen perfusion the atrium was dissected and a thin strip (25–40 mg of tissue) removed for study. One end of the strip was affixed to two 30-gauge platinum electrodes on a glass muscle mount. The other end was sutured to a fine gold chain leading to a model UL-5 Statham isometric transducer. The muscles were stimulated at threshold voltages (1-msec square-wave pulses) by a Tektronix Type 161 pulse generator. Isometric contractions at a rate of 30/min were recorded on a calibrated

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Received from the Departments of Anesthesiology and Pharmacology, University of Texas, Southwestern Medical School at Dallas, Dallas, Texas. Accepted for publication October 13, 1971. Supported by Research Grant GM-14439 and Training Grant GM-1421 from the National Institutes of Health. Presented at the 51st Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, New Jersey, April 1968

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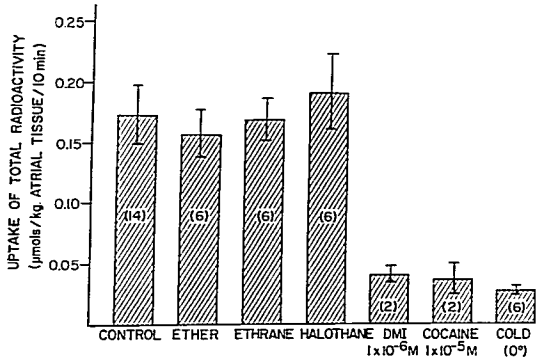

FIG. 1. Effects of volatile anesthetics on the uptake of total radioactivity of l-^{3}H-norepinephrine in guinea-pig atria. Number of experiments is shown in each bar graph. Uptake is expressed as mean ±SE.

Texas Instruments Oscillo/riter. The volume of the muscle chamber was 2 ml. The muscle was bathed in a modified Krebs-Henseleit solution of the following composition per liter (millimolar): Na^+ 145; K^+ 5.0; Ca^{++} 2.25; Mg^{++} 1.2; Cl^- 127; HCO_3^- 25; HPO_4^{--} 1.2; SO_4^{--} 1.2; glucose 10; EDTA 0.04. This solution was bubbled with 95 per cent oxygen–5 per cent carbon dioxide through a sintered glass disc so that there was fine bubble dispersion throughout the chamber. Temperature and *p*H were held constant at 37 C and 7.4, respectively.

After each atrial strip was fixed in the muscle chamber, the resting tension was adjusted so that the developed tension was near the peak of the length–tension curve. All atria were then equilibrated for a total of 20 minutes before experiments were conducted. Volatile anesthetics were administered via a special in-line vaporizer. Diethyl ether, halothane (Fluothane), and Ethrane § were studied. Anesthetic vapor concentrations in the inflow line to the chamber were determined by gas chromatography. Volatile anesthetics were administered for 15 minutes in concentrations sufficient to depress peak developed tension during the isometric twitch to approximately 50 per cent of its control value. Atrial preparations used as controls for the volatile anesthetic series were given 95 per cent oxygen–5 per cent carbon dioxide. In other experiments cyclopropane was administered to the atria in concentrations of 25–50 per cent rather than in concentrations producing 50 per cent depression in twitch height. Because of the possibility of oxygen limitation in these experiments with cyclopropane, control atria for these studies were given carbogen (95 per cent oxygen–5 per cent carbon dioxide) plus 20 or 50 per cent nitrogen. A calibrated Matheson Mass flowmeter was used to administer these gases.

§ Kindly supplied by Mr. James F. Vitcha, Ohio Medical Products, Division of Airco.

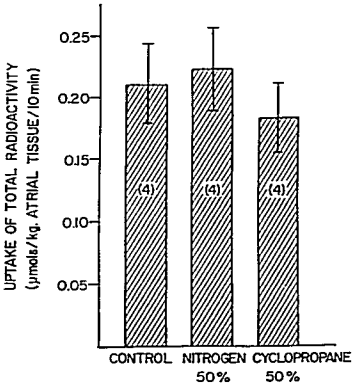

FIG. 2. Effects of nitrogen, 20 per cent, and cyclopropane, 25 per cent, on the uptake of total radioactivity of l-^{3}H-norepinephrine in guinea-pig atria. Number of experiments is shown in each bar graph. Uptake is expressed as mean ±SE.

After the 15-minute period allowed for equilibration with the anesthetics, *l*-^{3}H-norepinephrine was added in a final concentration in the bath of 1×10^{-7} M. Two minutes later the maximal inotropic response was recorded. After 10 minutes of contact with radioactive norepinephrine, the tissues were quickly washed in two changes of 0.9 per cent sodium chloride, removed from the bath, and blotted dry with Whatman no. 1 filter paper. The tissues were weighed and frozen for assay. The rate of uptake of *l*-norepinephrine was linear during the 10-minute exposure period and was believed to be a good approximation of the true initial rate of uptake.

The atria were ground with 5 ml of 5 per cent trichloroacetic acid in a glass homogenizer. The tubes were rinsed twice with 5 ml of 5 per cent trichloroacetic acid to give a final volume of 15 ml. This was centrifuged for 15 minutes at $10,000 \times g$ and the supernatant fluid decanted. The trichloroacetic acid fractions were divided and assayed for total radioactivity, ^{3}H-norepinephrine, and deaminated ^{3}H-metabolites, as described previously.[5]

Results (Figs. 1–3)

Depression of Contractility

Concentrations of volatile anesthetics necessary to produce equivalent (50 per cent) reductions in peak developed tension are shown in table 1. The effects of cyclopropane and nitrogen on atrial contractility are shown in table 2.

Effects of Halothane, Diethyl Ether, and Ethrane on Uptake of *l*-^{3}H-Norepinephrine

Figure 1 shows that there were no differences between the initial rates of uptake of total radioactivity in the anesthetized tissues and the control tissues. In this figure the effects of 1×10^{-6} M desmethylimipramine and 1×10^{-5} M cocaine are also shown. Since these agents are classic inhibitors of norepinephrine uptake, this figure illustrates that the system was capable of detecting alterations in the rate of catecholamine uptake.

Table 3 shows that the amount of total radioactivity present in the tissues as ^{3}H-norepinephrine was in every case greater than 90 per cent. Also, the percentages present as deaminated metabolites in control tissues and in those exposed to anesthesia were the same, suggesting that these volatile anesthetics do not alter the activity of intraneuronal monoamine oxidase.

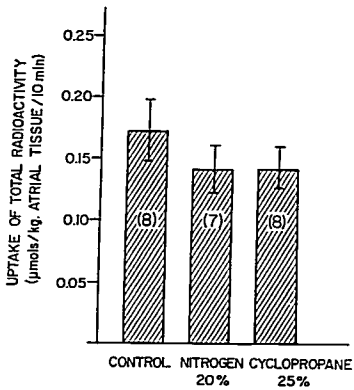

FIG. 3. Effects of nitrogen, 50 per cent, and cyclopropane, 50 per cent, on the uptake of total radioactivity of *l*-^{3}H-norepinephrine in guinea-pig atria. Number of experiments is shown in each bar graph. Uptake is expressed as mean ±SE.

Discussion

The termination of the physiologic action of both endogenous and exogenously administered norepinephrine takes place via the active uptake process of the amine into the adrenergic neuron.[6, 7] There has been speculation as to whether the sensitizing effect of cyclopropane on epinephrine-provoked ar-

TABLE 1. Anesthetic Concentrations Needed to Produce Equivalent Depression of Isometric Contractile Force in Guinea-Pig Left Atria

	Number Tested	Concentration (v/v in Vapor Phase) ± SE	Depression of Peak Developed Tension (Per Cent of Control) ± SE
Diethyl ether	6	6.40 ± 0.2	50 ± 4.6
Halothane	6	0.99 ± 0.02	52 ± 2.6
Ethrane	6	2.10 ± 0.2	52 ± 2.2

TABLE 2. Effects of Cyclopropane and Nitrogen on Myocardial Contractile Force in Isolated Guinea-pig Left Atria

	Number Tested	Concentration (v/v) ± SE	Depression of Peak Developed Tension (Per Cent of Control) ± SE
Cyclopropane	4	25 ± 1.3	20 ± 8.0
Nitrogen	4	20	9 ± 6.0
Cyclopropane	4	51 ± 1.3	82 ± 4.4
Nitrogen	4	50	47 ± 11.8

TABLE 3. Effects of Volatile Anesthetics on the Metabolism of l-^{3}H-Norepinephrine in Isolated Guinea-pig Left Atria

	Number Tested	Per Cent of Total Radioactivity Present as	
		^{3}H-Norepinephrine ± SE	^{3}H-Deaminated Metabolites ± SE
No anesthetic	14	92 ± 3.5	6 ± 0.6
Diethyl ether, 6.4 per cent	6	94 ± 3.7	6 ± 0.6
Halothane, 0.9 per cent	6	93 ± 3.7	5 ± 0.6
Ethrane, 2.1 per cent	6	100 ± 3.7	5 ± 0.6

TABLE 4. Effects of Cyclopropane and Nitrogen on the Metabolism of l-^{3}H-Norepinephrine in Isolated Guinea-pig Left Atria

	Number Tested	Per Cent of Total Radioactivity Present as	
		^{3}H-Norepinephrine ± SE	^{3}H-Deaminated Metabolites ± SE
No anesthetic	14	92 ± 3.5	6 ± 0.6
Nitrogen, 20 per cent	7	93 ± 6.7	7 ± 0.5
Cyclopropane, 25 per cent	8	89 ± 1.3	8 ± 0.9
No anesthetic	4	89 ± 3.2	8 ± 0.9
Nitrogen, 50 per cent	4	94 ± 3.2	7 ± 0.7
Cyclopropane, 50 per cent	4	93 ± 3.2	8 ± 0.7

rhythmias is in fact due to altered amine handling at the postganglionic adrenergic neuron. Price and Price,[5] working with the rabbit aortic strip (which unlike the heart contains predominantly α-adrenergic receptors), found that the dose–response curve to norepinephrine was shifted to the left in the presence of cyclopropane. This enhancement suggests a cocaine-like effect—*i.e.*, inhibition of norepinephrine uptake by adrenergic neurons. However, these investigators also showed that the curves for histamine, 5-hydroxytryptamine, and angiotensin were shifted to the left in the presence of cyclopropane, suggesting a direct action of the anesthetic on vascular smooth muscle. Since inhibition of uptake is the only well-defined mechanism that produces leftward shift of norepinephrine dose–response curves, the present experiments were designed to examine this possibility.

The results indicate that none of the anesthetics studied altered the initial rate of uptake of the adrenergic neurotransmitter, norepinephrine. These data confirm, with the physiologic *l* isomer of norepinephrine, previous suggestions by others.[1, 2] Two of the anesthetics studied, cyclopropane and halothane, augment the heart by lowering the arrhythmia threshold to injected catecholamines. It is highly unlikely that inhibition of uptake of norepinephrine is a mechanism of "sensitization" of the myocardium to catecholamines by these anesthetics. Price[9] and Price *et al.*[10] demonstrated that plasma catecholamine levels are increased during anesthesia with diethyl ether and cyclopropane. These elevated levels could be due to increased release, decreased uptake, decreased metabolism, or a combination of any of the three mechanisms. The data presented in this paper indicate that impairment of catecholamine uptake is not a major factor in the increased levels of these substances observed during anesthesia with cyclopropane and diethyl ether.

The deaminative metabolism of norepinephrine which occurs via intraneuronal mitochondrial monoamine oxidase (MAO) is likewise not altered by dosages of anesthetics which produce severe (50 per cent) negative inotropic effects. This would indicate that in-

creases of plasma norepinephrine which occur during anesthesia with anesthetics such as cyclopropane are not the result of impaired deaminative metabolism of the catecholamine. This is interesting in view of the fact that hepatic microsomal NADPH–oxygen-dependent oxidative metabolism of many drugs is depressed by halothane.[11, 12] However, these two metabolic reactions occur via different enzyme systems and require different cofactors, which again infers a certain degree of specificity to the action of anesthetics.

These findings concerning the uptake and metabolism of norepinephrine emphasize the select nature of the actions of inhalation anesthetics. Certain membrane and enzyme functions (*i.e.*, norepinephrine uptake and intraneuronal deamination) are quite unaffected by anesthetics in concentrations which severely depress other functions such as cardiac contractility.

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Respiration

ASPIRATION PNEUMONITIS Aspiration of gastric juice was found to have taken place on 25 occasions in 18 patients; seven were comatose, six had ileus, and five had anesthetic accidents. There was an inverse ratio between *p*H of the fluid aspirated and the associated symptomatology. All patients who aspirated fluid with *p*H values less than 1.75 died. Fluid above *p*H 2.4 did not produce pneumonitis. Hypotension occurred in proportion to the plasma volume deficit (as much as 1,200 ml) and the intrapulmonary right-to-left shunt resulted in a low Pa_{O_2}. Transient hypercarbia occurred in 12 patients. Oxygenation improved with IPPB, but three patients needed continuous positive-pressure breathing. Lung compliance was low, and remained low even after initial clinical, blood-gas, and x-ray evidence of improvement. Fifteen patients developed infections (six gram-positive; six gram-negative; three mixed) in spite of prophylactic antibiotic administration. Ten patients died; seven (40 per cent) as a direct consequence of aspiration pneumonia. Patients who died were twice as old as those who survived. (*Lewis, R. T., Burgess, J. H., and Hampson, L. G.: Cardiorespiratory Studies in Critical Illness, Arch. Surg. 103:335–340, 1971.*)