Effects of Halothane on Plasma Catecholamines

Michael F. Roizen, M.D.,* Jonathan Moss, Ph.D.,† David P. Henry, M.D.,‡
Irwin J. Kopin, M.D.§

The effects of halothane anesthesia on plasma catecholamines were studied in the rat utilizing a new, sensitive, radiometric assay. Total catecholamine levels in plasma during halothane (1 per cent) anesthesia were only about 12 per cent of those in awake animals. Plasma norepinephrine concentrations during anesthesia with 1 per cent halothane were half those found after recovery from anesthesia. Halothane also caused these changes in corticosterone-treated adrenalectomized rats. In intact rats, as the concentration of halothane increased to 1.5 per cent, plasma total catecholamine levels fell further, paralleling a fall in blood pressure. This depression in catecholamines was found to be the result of neither the duration of anesthesia nor the amount of blood taken. The study suggests that the cardiovascular effects of halothane are associated with halothane-induced changes in sympathetic neuronal activity. (Key words: Anesthetics, volatile; halothane; Sympathetic nervous system: catecholamines.)

EGER AND ASSOCIATES¹ have reported that in man anesthesia with 1 per cent halothane decreases cardiac output, stroke volume, mean arterial pressure, left ventricular minute and stroke work, and myocardial contractility, while increasing right atrial pressure. Among possible factors that would explain these changes is sympathetic nervous system depression, but prior investigations of this point have been inconclusive. Price et al.² reported that halothane produced no consistent change in either serum norepinephrine or epinephrine concentration.

They found increases in catecholamine levels (as measured by the trihydroxyindole method) when respiratory acidosis was allowed to develop during halothane anesthesia, but their initial values were at the lower limits of sensitivity for their assay technique. Hamelberg et al.³ reported slightly increased concentrations of serum catecholamines in man during surgery with “light” halothane anesthesia and premedication, but no statistically significant increase during surgery with “deep” halothane anesthesia. Anton et al.⁴ found increased catecholamines in blood and urine during heart surgery with halothane anesthesia. However, these studies, however, were done with the relatively insensitive fluorometric assay for catecholamines.

Halothane has not been found to have striking effects on the sympathetic nervous systems of animals. Ngai et al.⁵ found that halothane had no effect on uptake of tritiated norepinephrine by rat heart or release of labelled norepinephrine from dog heart. Naito and Gillis⁶ found halothane had no effect on the in-citro uptake of labelled norepinephrine by cat atra. In rats, halothane anesthesia had no effect on norepinephrine synthesis in heart or brain. Skovsted et al.⁷ showed that halothane produces only slight depression of sympathoadrenal activity induced by baroreceptor nerve stimulation in normal, decerebrated, or spinal cats.

While clinical observations and physiologic data suggest that halothane might have an effect on the sympathetic nervous system, experimentally it has been difficult to demonstrate convincing changes in adrenergic activity. The relative insensitivity of the fluorometric technique has precluded demonstration of decreases in catecholamine levels, which are barely detectable by fluorometry. The sensitivity of radioisotopic enzymatic techniques provides the required sensitivity. Using such methods, we have found striking decreases in plasma catechol-
amines in both normal and adrenalecto-
mized rats anesthetized with halothane.

Methods

Normal male Sprague-Dawley rats weighing 250 to 300 g, or adrenalectomized rats (all obtained from Hormone Assay Laboratories, Chicago, Illinois) maintained on 1.5 mg/kg corticosterone administered (daily) by subcutaneous injection, were allowed to acclimate to their new surroundings for 4–7 days, with numerous handlings by the inves-
tigators. Anesthesia was induced by introduc-
ing through an inlet in a plexiglas container 3 l/min of 3 per cent halothane (Fluothane, Ayerst) in oxygen. After 5 minutes of exposure to 3 per cent halothane, anesthesia was maintained with a 1 per cent halothane/ oxygen mixture introduced by face mask. The left carotid artery was exposed through a vertical incision in the throat and cannulated with PE-90 tubing, which was exteriorized at the back of the neck, and the incision closed with clips. The catheter was filled with a 50 per cent (v:v) solution of Rheo-Macrodex (Pharmacia, New Jersey) containing 1,500 units of heparin sulfate per ml. The animals were then transferred back to the induction box and allowed to breathe spontaneously 1 per cent halothane in oxygen. After 30 minutes, 0.6 ml arterial blood was removed and placed in a heparinized tube. The volume of blood removed was replaced with physiologic saline solution and the catheter refilled with Rheo-Macrodex/heparin sulfate. The rat was then allowed to awaken, and 4 to 6 hours later (and after five to ten handlings to acclimate the animal), 1.0 ml blood was taken, the volume of blood removed again being replaced with physiologic saline solution. One to three weeks later, these animals were decapitated after many handlings, and blood collected from the bleeding neck into heparinized beakers.

All blood samples were immediately cooled on ice and centrifuged at 6,000 x g for 20 minutes. The proteins in 300 µl of the serum were precipitated by addition of 5 µl concentrated (60 per cent) perchloric acid, and the acidified samples were frozen and kept at -10 °C until assayed for catechol-
amines as described below. An additional sample of plasma was also frozen and kept at -10 °C until assayed for dopamine-beta-hydroxylase.

In a second set of experiments, normal male Sprague-Dawley rats weighing 250–300 g were obtained from Hormone Assay Laboratories (Chicago, Illinois). The animals were allowed to acclimate to their new surroundings for 4–7 days. Anesthesia was induced as before and maintained with a face mask until a tracheostomy using a 16 An-
agoch (Becton-Dickinson) was performed. Each rat was then mechanically ventilated 50 times per minute with 1 per cent halothane in oxygen, using a tidal volume of 3 ml delivered by a Bird-Phillips small-animal respirator (Richmond, Virginia).

The mean blood pressure in the right femoral artery was recorded using PE-60 polyethylene cannula (Clay-Adams) connected to a Statham blood-pressure trans-
ducer and a Sanborn Polysino polygraph. The right carotid artery was cannulated with a PE-90 cannula for blood collection; the right jugular vein was cannulated with PE-90 tubing for blood volume replacement and heparin administration. Immediately after the last cannulation was completed, heparin sulfate (300 IU) (The Upjohn Company, Kalamazoo, Michigan) was administered intravenously, and after 30 minutes, 0.6 ml of blood from the carotid catheter was rapidly collected in a heparinized tube; an equal volume of physiologic saline solution (Abbott, Chicago, Illinois) was then infused via the jugular cannula during a 3-minute interval. After the blood pressure returned to baseline, the halothane concentration was increased to 1.5 per cent for an additional 30 minutes before a second sample of blood was ob-
tained. After replacement of the 0.6-ml blood sample with an equal volume of physio-
logic saline solution, the blood pressure was allowed to return to baseline before the halothane concentration was decreased back to 1 per cent in oxygen. After another 30 minutes of anesthesia, another 0.6-ml blood sample was collected, along with 0.6 ml for blood pH analysis. pH was measured with a glass electrode (Radiometer, Copenhagen) and only blood samples of animals whose final blood pH's were in the range of 7.33 to
7.47 were used in the study. (Bloods from 4 of 16 animals were discarded because pH's of the final blood samples were more (three animals) or less (one animal) than this range.) Blood samples were prepared as described above.

ASSAY FOR TOTAL CATECHOLAMINES

The method used was a modification of that of Coyle and Henry.9 The frozen perchloric acid–plasma samples were thawed and centrifuged at 6,000 × g at 4 C for 20 minutes. Then, 25-μl samples of clear supernatant were added to 15-ml glass-stoppered centrifuge tubes containing either 50 μl of 0.1 N perchloric acid or 500 pcp (free base) of norepinephrine dissolved in 50 μl of 0.1 N perchloric acid. Blanks consisted of 75 μl of 0.1 N perchloric acid for assay. The enzymatic reaction was initiated by addition of 35 μl of a mixture containing 0.3 mg of dithiothreitol, 3 μl of 0.1 M MgCl₂, 35 μl of 2 M tris–HCl buffer, pH 9.6, 6 μl of catechol-O-methyltransferase solution containing 0.3 m units (purified and standardized by the method of Axelrod and Tomchick9), 9 μl of distilled water and 1.7 μCi of [3H-methyl]-S-adenosylmethionine (specific radioactivity 8.5 mCi/μmol; New England Nuclear Corp., Boston, Massachusetts). The mixture was incubated for 90 minutes at 37 C and the reaction stopped by the addition of 500 μl of 0.5 M borate buffer (pH 10).

After the addition of nonradioactive carriers (7 μg of methoxytyramine, 3 μg of normetanephrine, and 3 μg of metanephrine), the O-methylated products were extracted into water-saturated ethyl acetate:methanol (10:1, v/v) by shaking for 5 minutes on an Eberbach reciprocating shaker (Ann Arbor). The phases were separated by centrifugation at 200 × g for 2 minutes and 8.5 ml of the organic phase was transferred to another tube and washed with 0.5 ml of 0.5 M borate buffer (pH 10); 7.5 ml of the organic phase were then shaken with 0.5 ml of 0.1 N HCl for 5 minutes. The phases were separated by centrifugation at 200 × g for 2 minutes and the organic phase was aspirated and discarded. The aqueous phase was washed with 9 ml of water-saturated ethyl acetate, and the organic phase discarded. The assay was then completed as outlined by Coyle and Henry.9 Standard curves were derived by adding authentic catecholamines to plasma obtained by decapitation of both normal and hexamethonium-treated rats.

ASSAY FOR NOREPIINEPHRINE

This assay was modified from those of Saelens11 and Iverson and Jarrott.12 For assay of norepinephrine another sample (60 μl) of the clear supernatant from the perchloric acid-treated plasma was placed into iceed 15-ml glass-stoppered centrifuge tubes containing 150 μl of a 0.01 N hydrochloric acid solution, or 250 pcp (free base) of norepinephrine dissolved in 150 μl of 0.01 N hydrochloric acid solution. Blanks consisted of 60 μl of a 0.2 N perchloric acid solution combined with 150 μl of a 0.01 N hydrochloric acid solution.

The enzymatic reaction was initiated by addition of 50 μl of a mixture containing 0.5 mg dithiothreitol, 2 mg EDTA, and 0.08 mmol tris–HCl buffer, pH 8.6, 3.4 μCi of [3H-methyl]-S-adenosylmethionine (specific radioactivity 8.5 mCi/μmol; New England Nuclear Corp., Boston, Massachusetts), and 6 μl of a solution of phenylethanolamine-N-methyltransferase (specific activity 76.7 nmol of N-methylated phenylethanolamine formed/mg protein/hour, prepared as described by Molinoff, Weisman, and Axelrod.13) The reaction was incubated for 90 minutes at 37 C in a Dubnoff metabolic shaking incubator. The reaction was stopped by placing the extraction tubes on ice and adding 2 ml of 2 M tris–HCl and 0.5 M phosphate buffer, pH 8.6, containing 5 per cent EDTA. Catecholamines were adsorbed onto 100 mg of alumina by mixing on a Vortex “genie” for 15 seconds. The alumina was separated by centrifugation at 200 × g for 2 minutes, and the supernatant aspirated and discarded. The alumina was then washed three times with H₂O, and the catecholamines were eluted by adding 1 ml of 0.1 N perchloric acid. The supernatant was transferred to 3-ml tubes on an ice bath. To remove residual [3H-methyl]-S-adenosylmethionine, 200 μl of freshly prepared...
25 per cent phosphotungstic acid solution and 100 µl of a solution containing 0.5
mg/ml epinephrine and 1 mg/ml of non-
radioactive S-adenosyl-methionine in 0.2 N
acetic acid were added. After 15 minutes in
the cold, the mixture was centrifuged at 4 C
and 8,000 x g for 10 minutes. Immediately
thereafter, the supernatant was transferred to
extraction tubes containing 1 ml of 1 M
potassium phosphate buffer (pH 7.15). The
radioactive end product was extracted in 10
ml of 1 per cent di-(2-ethylhexyl)-phosphoric
acid in toluene; 9 ml of the organic phase
were transferred to a counting vial containing
400 µl liquifluor (New England Nuclear Corp.)
and tritium assayed by liquid scintillation
spectrometry. All samples were assayed
in duplicate with internal standards for the
catecholamine assays.

![Graph 1: Relationship of recovered ^3H-O-
methylated derivatives of catecholamines and the
amounts of epinephrine and norepinephrine added
to plasma from a hexamethonium-treated rat
(Plasma H) and an untreated rat (Plasma N). The
amounts of catecholamines added to Plasma N
were: 7 ng/ml (2 ng norepinephrine and 5 ng
epinephrine); 15 ng/ml (5 ng norepinephrine and
10 ng epinephrine); 25 ng/ml (10 ng norepineph-
rine and 15 ng epinephrine); or 25 µg/ml epine-
phrine (open circle). Only norepinephrine was
added to Plasma H. The O-methylated derivatives
were formed enzymatically from ^3H-methyl-S
adenosyl-methionine by the action of catechol-O-
methyltransferase, as described in Methods. The
Blank of 39 cpm was subtracted from each value.

![Graph 2: Relationship of measured ^3H-epinephrine
formed from norepinephrine added to plasma from
a hexamethonium-treated rat. The radioactive
compound was formed enzymatically from ^3H-
methyl-S-adenosylmethionine by the action of
phenylethanolamine-N-methyltransferase, as
described in Methods. The blank of 380 cpm was
subtracted from each value.

Standard curves were prepared by addition
of authentic catecholamines to plasma ob-
tained by decapitation of both normal and
hexamethonium-treated rats.

Dopamine-beta-hydroxylase was measured
in duplicate samples by the method of
Molinoff, Weinsilboum and Axelrod.13

| TABLE 1. Recovery of Total Catecholamines (CA)
and Norepinephrine (NE) Added to Rat Plasma* |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmal</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>H</td>
</tr>
<tr>
<td>H</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td>N</td>
</tr>
</tbody>
</table>

* Values for norepinephrine and total CA were determined from the
radioactivity measured in the methylated derivatives, as
described in Methods.

1 Plasma H was obtained from a rat treated with hexamethonium
(10 mg/kg ip) one hour before decapitation, while Plasma N
was obtained from an untreated rat.
FIG. 3. Plasma catecholamines in anesthetized and awake animals. Halothane (1 per cent) anesthesia was induced as described in Methods, and blood samples were obtained from the arterial cannula after 30 minutes of anesthesia and 4–6 hours after the animals awoke from anesthesia. Three weeks later blood was obtained after decapitation. Results are the mean values (±SEM) for groups of 8–11 animals. The plasma levels of both total catecholamines and norepinephrine in anesthetized animals were significantly less \( P < 0.01 \) and \( P < 0.02 \) than in either group of awake animals.

**Calculations**

Values for plasma norepinephrine were determined using the phenylethanolamine-N-methyltransferase procedure, using a correction for recovery as indicated by use of internal standards. The values for norepinephrine were used to calculate the contribution of this catecholamine to the tritiated O-methylated derivatives recovered in the assay using catechol-O-methyltransferase. The tritiated compounds in excess of this were attributed to methanephrine, the O-methylated derivative of epinephrine. A correction was made for recovery of epinephrine (which is more efficiently measured, by about 25 per cent, than is norepinephrine).

**Results**

The method for assay of norepinephrine and total catecholamines was sufficiently sensitive to measure less than 25 pg of norepinephrine and less than 50 pg of total catecholamines (epinephrine and norepinephrine). Thus, these amines in plasma of rats, even after procedures which result in striking decreases in their concentrations, such as ganglionic blockade or adrenalectomy, could readily be measured. There was a linear relationship between the amount of catecholamine added to plasma and the tritiated methylated derivatives measured (figs. 1 and 2). Recoveries ranged from 87 to 113 per cent (table 1).

The catecholamine content of plasma obtained from rats anesthetized with halothane was much lower than that in plasma obtained from the cannulas of the same animals after recovery from anesthesia (fig. 3). The mean (±SEM) total catecholamine (epinephrine and norepinephrine) concentration was 1.45 (±0.31) ng/ml in plasma of 11 animals anesthetized with 1 per cent halothane, while in 11 awake animals it was 11.94 ± 3.46 ng/ml. Norepinephrine accounted for only a small portion (1.95 ± 0.46 ng/ml) of the total catecholamine content of plasma of awake animals.

**Fig. 4.** Plasma catecholamines in anesthetized and awake adrenalectomized rats. Halothane anesthesia was induced and blood samples obtained as described in the legend to figure 3 and in Methods. Results are the means (±SEM) for groups of 5–10 animals. The plasma levels of both total catecholamines and norepinephrine in anesthetized animals were significantly less \( P < 0.001 \) than in either group of awake animals.
animals (table 1, fig. 3), but levels of this catecholamine were also strikingly reduced (0.68 ± 0.09 ng/ml) in plasma of the anesthetized rats. The catecholamine content of plasma obtained from blood collected after decapitation of eight animals was not significantly higher than that of plasma obtained from blood collected from indwelling cannulas of the animals when awake (fig. 3).

The total catecholamine content of plasma obtained from the cannulas of 10 awake adrenalectomized rats was, as expected, lower than that of normal animals (fig. 4). During halothane anesthesia the catecholamine levels of the adrenalectomized rats were lower than those found in these animals after recovery from anesthesia. After decapitation, levels were slightly higher ($P < 0.5$) than levels in plasma obtained from cannulas of 5 awake adrenalectomized rats (fig. 4).

During halothane anesthesia, the plasma levels of catecholamines paralleled the blood pressure changes (fig. 5). Increasing the concentration of halothane from 1.0 per cent to 1.5 per cent for 30 minutes resulted in decreases of blood pressure and plasma catecholamines. When the concentration of halothane was returned to 1.0 per cent for 30 minutes, both plasma catecholamines and blood pressure returned to their previous levels.

In neither set of experiments did anesthesia affect plasma dopamine-beta-hydroxylase activity.

**Discussion**

Halothane anesthesia depressed arterial catecholamine levels to below the levels found in awake, air-breathing animals. The eightfold reduction in combined norepinephrine and epinephrine (dopamine levels in all cases were below 0.1 ng/ml, the lower limit of sensitivity of the assay) was accompanied by a threefold depression in serum norepinephrine content. When the inspired halothane concentration was increased from 1 to 1.5 per cent, the decline in serum catecholamines paralleled the decline in blood pressure. These changes in plasma catecholamines and blood pressure did not appear to be time-related, since both returned to their initial values after an additional 30 minutes of anesthesia with 1 per cent halothane (fig. 5). Furthermore, the fact that circulating catecholamines and blood pressure in intact rats did not vary significantly between the first and third samples indicates that withdrawal of the first two samples of blood did not influence the level of catecholamines in the third sample.

The lower catecholamine content of plasma of adrenalectomized animals is consistent with the observation that most of the circulating catecholamines in normal intact animals is epinephrine. In adrenalectomized rats as well as in normal rats, halothane
anesthesia resulted in reduction of plasma catecholamines, indicating that this anesthetic agent reduces sympathetic neuronal as well as adrenal medullary release of catecholamines.

In man, the effects of halothane anesthesia on circulating catecholamines are unclear. Price et al. could not demonstrate a decrease in catecholamines in plasma, perhaps because the fluorometric method used in these studies could barely detect levels of catecholamines in plasma of awake patients. Hamelberg et al. used a similar method to measure catecholamines; however, the patients examined had received a variety of preanesthetic medications and were subjected to surgical procedures. In their study peripheral venous blood was used. We have found that the levels of catecholamines in plasma of venous blood obtained from the antecubital veins of anesthetized patients undergoing cardiac surgery bear little relationship to the levels of catecholamines in plasma of arterial blood (unpublished observations). The venous blood obtained from the antecubital vein may be derived from superficial veins of the skin and reflect local changes in sympathetic nerve activity. Anton et al. found increased catecholamines in plasma of halothane-anesthetized patients during surgery, but many premedications were given and thoracotomies were performed.

The levels of catecholamines in the plasma obtained from blood withdrawn from arterial canulas of awake rats were similar to the values found in the same animals three weeks later when blood was obtained after decapitation. Both these values were higher than those found in anesthetized animals. Similar results were obtained in adrenalectomized rats, although the levels of catecholamines in plasma of the awake adrenalectomized rats were lower than those in intact animals. It is not possible to exclude the effects of stress resulting from handling of the awake animals, but the animals did not appear to be disturbed by handling during blood withdrawal from implanted canulas. Certainly stress-induced phenomena could not account for the dose-related changes in systemic catecholamines in anesthetized animals (see fig. 5).

Depression by halothane of sympathetic outflow is consistent with the cardiovascular changes seen during halothane anesthesia: decreases of cardiac output, stroke volume, mean arterial pressure and myocardial contractility. It appears unlikely that the 90 per cent reduction in epinephrine and the 50 per cent decrease in serum norepinephrine totally account for the 25 per cent reduction in cardiac output and stroke volume seen after one hour of anesthesia with 1 per cent halothane. It is interesting, however, to observe that the relative decrease in plasma catecholamines parallels the decrease in ganglionic transmission associated with halothane anesthesia. In 1956, Raventos reported that transmission through the mesenteric ganglion was more easily depressed by halothane than was transmission through the superior cervical ganglion. Assuming epinephrine arises mainly from the adrenal medulla, our findings of a 90 per cent decrease in epinephrine concentration in plasma from awake compared with halothane (1 per cent)-anesthetized rats suggest that halothane acts on adrenal medullary release to a greater extent than it does on sympathetic neurons. The 50 per cent depression in norepinephrine concentration closely parallels the 35 to 50 per cent depression by halothane of neuronal activity in the cervical sympathetic trunk of intact cats. The parallel decreases in norepinephrine levels and sympathetic neuronal activity suggest that the amounts of norepinephrine released per nerve impulse are similar regardless of the rate of stimulation. At low, physiologic, frequencies of nerve stimulation, levels of norepinephrine entering the circulation are too low to measure and to correlate with frequency of stimulation. The amount of norepinephrine released per nerve impulse from the perfused cat spleen increases with increased rates of stimulation, reaching a maximum at 30 impulses, but in the presence of dibenamine the amounts of norepinephrine released per nerve impulse are similar regardless of frequency of stimulation.

The mechanism of decreased sympathetic activity during halothane anesthesia appears, at least in part, to involve central mechanisms controlling the peripheral sym-
pathetic nervous system. The decrease in plasma catecholamines with halothane anesthesia appears to be a result of diminished catecholamine release from the adrenal medulla, as well as norepinephrine from the sympathetic nerves.

ADDENDUM

While this report was being reviewed for publication, Perry, Van Dyke, and Theye (Anesthesiology 40:463-70, 1974) reported that in dogs halothane anesthesia lowered both mean arterial blood pressure and plasma levels of both epinephrine and norepinephrine. The "awake" animals which were used as controls were treated with "minimal amounts of thiopental 1 hour after completion of the third week of study with an anesthetic agent in each dog." We have found that barbiturates also lower plasma catecholamine levels (unpublished observations); thus, the values reported by Perry et al., for "awake" dogs may be too low. The values for dog plasma norepinephrine reported by Anton and Sayre (J Pharmacol Exp Ther 138:160-173, 1962) are nearly five times as great as those of Perry, but epinephrine values were lower. In most species, except the rat, norepinephrine levels normally are greater than epinephrine levels unless adrenal medullary discharge has been stimulated. There is, however, agreement that during halothane anesthesia there is a reduction in sympathoadrenal medullary discharge with a concurrent decrease in arterial pressure.

References