Effects of Anesthetics on Myocardial Catecholamines

Tsung-Han Li, M.D., Lembt Hans Laasberg, Ch.E., Benjamin E. Etsten, M.D.

In nonreserpinized dogs the catecholamine content was greater in the atrial than in ventricular tissue; the atrial-ventricular ratio was approximately 1.8:1 for epinephrine and 2:1 for norepinephrine. The ratio of norepinephrine to epinephrine in atrial and ventricular tissues was 10:1. In nonreserpinized dogs, the catecholamine contents of cardiovascular tissues were not significantly changed following thiopental anesthesia, but norepinephrine content was significantly increased during ether and cyclopropane anesthesia in contrast to the significant depletion of epinephrine following ether, cyclopropane and halothane, in that order of magnitude.

In the reserpinized group before anesthesia, 95% per cent of norepinephrine stores were depleted, with some reduction in epinephrine. Anesthetics exerted no effect upon the catecholamine contents of reserpinized animals. The unaltered tissue epinephrine content of the reserpinized dogs was in strong contrast to the depletion after ether and cyclopropane in nonreserpinized dogs. The mechanisms of these changes are discussed with reference to the hemodynamic effects of anesthetic agents.

The concept of the changes in concentration of circulating plasma catecholamines has been widely invoked to explain the various circulatory effects of general anesthetic agents. Plasma concentrations, however, do not serve as an infallible criterion for the activity of the sympato-adrenal system. The dilution of the minute quantities of liberated catecholamines by the circulating blood volume precludes this theory. Furthermore, a large quantity of catecholamines is erratically liberated from the adrenal medulla into the systemic circulation. Portions of the minute amounts of catecholamines set free at postganglionic adrenergic nerve endings or chromaffin tissues are combined at the receptor site, and the residual portions are mixed with larger quantities of catecholamines liberated from the adrenal glands. Therefore, the changes of circulating plasma catecholamines represent the combined processes of biosynthesis, liberation, binding and metabolism occurring at various body sites.

The importance of the storage portion of catecholamines in the myocardial and vascular tissues in maintaining circulatory homeostasis has been described recently. In view of this, the study of the influence of anesthetic agents upon the tissue concentration of catecholamines was considered a more rational approach in explaining the circulatory changes during anesthesia.

This communication describes changes in the levels of catecholamines in left and right atria, left and right ventricles, pulmonary artery and aorta following the administration of various anesthetic agents in nonmedicated and in reserpinized animals.

Method

Sixty-one mongrel dogs were divided into two major groups: 45 nonreserpinized and 16 reserpinized.

Nonreserpinized. This group was divided into five subgroups: (1) control, nonpremedicated and nonanesthetized, (2) thiopental, (3) ether, (4) cyclopropane, and (5) halothane.

Each subgroup consisted of 9 dogs. The same experimental procedures were used but the anesthetic agents differed in each subgroup. The control subgroup was studied under local infiltration anesthesia with 1 per cent procaine.

The dogs were intubated for controlled respiration following the intravenous administration of succinylcholine. Under 1 per cent procaine infiltration anesthesia, one femoral artery was exposed and cannulated for recording of the arterial blood pressure and for withdrawing arterial blood samples for analysis of blood gases. Statham P23D or P23G pressure transducers were used to obtain arterial pressures. Lead 2 of the electrocardiogram

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Table 1. Recovery of Catecholamines Control Standards Through Extraction and Identification Procedures

<table>
<thead>
<tr>
<th>Added µg</th>
<th>Recovered µg</th>
<th>Percentage Recovery</th>
<th>Added µg</th>
<th>Recovered µg</th>
<th>Percentage Recovery</th>
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<tbody>
<tr>
<td>0.1</td>
<td>0.08</td>
<td>80</td>
<td>0.1</td>
<td>0.84</td>
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<tr>
<td>0.1</td>
<td>0.11</td>
<td>110</td>
<td>0.08</td>
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<td>98</td>
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<tr>
<td>0.1</td>
<td>0.08</td>
<td>80</td>
<td>0.1</td>
<td>1.04</td>
<td>104</td>
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<tr>
<td>0.15</td>
<td>0.14</td>
<td>94</td>
<td>0.08</td>
<td>0.75</td>
<td>75</td>
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<tr>
<td>0.1</td>
<td>0.10</td>
<td>100</td>
<td>0.5</td>
<td>0.11</td>
<td>82</td>
</tr>
<tr>
<td>0.1</td>
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<td>100</td>
<td>0.75</td>
<td>0.61</td>
<td>62</td>
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<tr>
<td>0.4</td>
<td>0.45</td>
<td>111</td>
<td>1.5</td>
<td>1.07</td>
<td>107</td>
</tr>
<tr>
<td>0.4</td>
<td>0.39</td>
<td>97</td>
<td>1.5</td>
<td>1.12</td>
<td>93</td>
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<tr>
<td>0.2</td>
<td>0.17</td>
<td>85</td>
<td>1.5</td>
<td>1.27</td>
<td>85</td>
</tr>
<tr>
<td>0.2</td>
<td>0.17</td>
<td>85</td>
<td>1.0</td>
<td>1.05</td>
<td>105</td>
</tr>
</tbody>
</table>

$\bar{x} = 90.5\pm0.6\;\mu$  \hspace{1cm}  $\bar{x} = 91.4\pm0.6\;\mu$

and systemic arterial pressure were recorded on a multi-channel Sanborn direct writing recorder at paper speed of 1 mm. per second. One femoral vein was cannulated for the administration of drugs. Respiration was controlled with a volume-limited ventilator. Tidal volume and respiratory frequency were kept constant. Arterial blood gases were maintained within physiologic levels throughout the experiment.

Arterial blood samples were periodically taken for arterial blood pH and PCO$_2$ measured by means of a Sanz-Metrom capillary pH electrode and Severinghaus PCO$_2$ electrode with an Epsco Blood Parameter Analyzer. The pH meter was calibrated against three buffer solutions (National Bureau of Standards).

In the first subgroup (control) 100 per cent oxygen was administered via a nonrebreathing system for three hours. In the second subgroup, thiopental anesthesia was administered (total dose: 20–40 mg./kg.) intravenously at the rate of 5 mg./kg. per hour by infusion. In the third subgroup, ether anesthesia was administered via an E.M.O. ether vaporizer; inspiratory concentration was 4–15 ml./100 ml. for induction and maintained at 10–20 ml./100 ml. for three hours. In the fourth subgroup, cyclopropane anesthesia was administered using a closed-circuit rebreathing system; end expiratory concentration was 30–40 ml./100 ml. for induction and 15–25 ml./100 ml. for maintenance; the concentration of cyclopropane was determined by an absorption technique in 31 N sulfuric acid.$^{11}$ In the fifth subgroup, halothane anesthesia was administered via a Fluotec vaporizer; inspiratory concentration was 1.5–2.0 ml./100 ml. for induction and 1.0–1.5 ml./100 ml. for maintenance.

At the conclusion of a three-hour period of a steady state, the animals were killed by rapid intravenous injection of 200–300 ml. of air. The chest was opened quickly and the heart exposed. The right atrium, left atrium, right ventricle, left ventricle, aorta and pulmonary artery were rapidly incised and the blood quickly blotted away with gauze. These tissues were weighed separately and frozen in liquid nitrogen. They were homogenized in a mortar and the catecholamines were extracted with 0.4 N perchloric acid. The method of extraction was adapted from the procedure described by Bertler et al.$^{12}$ and Anton et al.$^{13}$ The acid extracts were kept at $-19^\circ$ C. overnight. After thawing, the perchlorate was precipitated by titration with 5 N potassium carbonate to pH 4; continuous stirring was used during titration. Potassium perchlorate was spun down by centrifugation and precipitates were washed with 1 ml. glass-distilled water. Washings were added to the catecholamine extracts.

Catecholamines were separated from extracts by using HCl washed alumina (Woelm, neutral, activity grade 1) as absorbent. Before alumina chromatography, the pH of extracts was brought up to 8.2 with K$_2$CO$_3$ and kept at this value during chromatography. The catecholamines were eluted from alumina with 0.3 N acetic acid. Aliquots from alumina eluate were taken for analysis and pH adjusted to 6.2 before oxidation. For estimation of catecholamines the trihydroxyindole (TH1) method$^{14}$ was used. Potassium ferricyanide served as the reagent to oxidize epinephrine and norepinephrine into indole derivatives, adrenochrome and noradrenochrome, respectively. By rearrangement with alkali (+ ascorbic acid) trihydroxyl indoles were formed. The resulting fluorescence was determined in an
Aminco-Bowman Spectrophotofluorometer at pH 5. Activation wave lengths were 450 and 405 millimicrons and fluorescence read at 535 millimicrons. The wave lengths reported here are uncorrected instrumental values. The content of epinephrine and norepinephrine was calculated by solving the two simultaneous equations.12-14

Reserpinized. Subgroups of 4 dogs each were studied as follows: (1) control, breathing 100 per cent oxygen, (2) thiopental anesthesia, (3) ether anesthesia, and (4) cyclopropane anesthesia.

The procedure of reserpinization consisted of the intramuscular injection of 0.5 mg./kg. body weight the first day; 0.5 mg./kg. of reserpine the second day; on the third day no further medication was given and the study was performed in the same manner as described for the nonreserpinized group.

Results

Accuracy of the Method. It is realized that the catecholamines in the tissues or in the body fluids usually consist of minute amounts of epinephrine with norepinephrine content approximately ten times greater than epinephrine. In order to detect minute quantities of epinephrine, therefore, the fidelity of the method used was checked against known quantities of epinephrine and norepinephrine in three different procedures. Known concentrations of epinephrine and norepinephrine were added to "blank" acetic acid eluates from alumina column and analyzed.

Table 1 presents the amount of catecholamine recovered after the known solutions were taken through the steps for extraction and identification. These results indicate that the variation of the recovered concentration of catecholamines is within the limits of technical error of recovery for epinephrine, 93.6-90.5 per cent, and for norepinephrine, 90.8-91.4 per cent.

Nonreserpinized Group: The mean concentration of the catecholamines found in the 5 subgroups are shown in table 2.

The values for myocardial catecholamine content of the control animals are shown in table 2 and figures 1 and 2. The content of norepinephrine was apparently ten times

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean±S.E. (Range)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right atrium</td>
<td>0.13±0.01 (0.02-0.21)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left atrium</td>
<td>0.11±0.01 (0.02-0.22)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Right ventricle</td>
<td>0.09±0.01 (0.02-0.17)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Left ventricle</td>
<td>0.07±0.01 (0.02-0.12)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Aorta</td>
<td>0.04±0.01 (0.01-0.09)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pulmonary artery</td>
<td>0.15±0.01 (0.02-0.30)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
greater than that of epinephrine in the myocardial tissues. The right and left ventricles contained less than one-half the concentration of norepinephrine found in the atrial tissues. The content of epinephrine in the right and left ventricles was one-half that obtained in the atrial. Considerable amounts of epinephrine were found in the pulmonary artery, more than double the amount of epinephrine in the aorta, although both arterial tissues had about the same amount of norepinephrine.

In three experiments the sino-atrial nodal region was excised for analysis of catecholamine content. The values were not different from those of atrial tissues. The values of myocardial catecholamines under thiopental

![Diagram](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931630/)

**Fig. 1.** Mean values (±2 × S.E.) of norepinephrine in cardiovascular tissues of anesthetized dogs compared with controls. R.A.: right atrium, L.A.: left atrium, R.V.: right ventricle, L.V.: left ventricle, A.: aorta, P.A.: pulmonary artery.

![Diagram](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931630/)

**Fig. 2.** Mean values (±2 × S.E.) of epinephrine in cardiovascular tissues of anesthetized dogs compared with control values.
anesthesia showed no statistically significant difference from the values obtained in the control group (table 2).

The most striking difference between the ether group and the control group (table 2) was the disappearance of epinephrine from the atrial ventricular tissues. These changes were statistically significant. Even the aorta and the pulmonary artery showed a tendency to lose epinephrine although the values were not statistically significant. With ether anesthesia, there was a significant increase in the content of norepinephrine in atrial and ventricular tissues and aorta.

The results from the cyclopropane group (table 2) were almost the same as found in the ether group. Epinephrine was significantly depleted (except left atrium) and the norepinephrine content was significantly increased in all myocardial tissues. The content of the catecholamines of the aorta and pulmonary artery showed the same tendency, although the results were statistically insignificant.

There was also significant depletion of epinephrine in the atrial tissues with halothane as shown in table 2. The most outstanding finding was that the norepinephrine content of all the cardiovascular tissues remained unaltered. Epinephrine contents of the ventricular tissue, the aorta and the pulmonary artery were not significantly different from the control group.

Results obtained from the control, ether, cyclopropane and halothane groups are charted in figure 1 (norepinephrine) and figure 2 (epinephrine).

**Reserpized Group:** Results are summarized in table 3. Since only 4 animals were studied and the changes were uniformly in the same direction, no attempt at statistical analysis was made. Reserpized dogs lost norepinephrine either completely or the content dropped from one-tenth to one-twentieth of normal values. It may be seen from table 2 that the norepinephrine contents were reduced to the level of the content of epinephrine in all four groups. However, the content of epinephrine was only partially reduced by reserpine. Anesthetics caused no change in the content of both epinephrine and norepinephrine in the reserpized dogs. Only thiopental, ether and cyclopropane anesthesia were studied. These results, including the control (nonreserpinized and reserpinized) are charted in figure 3 (norepinephrine) and figure 4 (epinephrine).

**Discussion**

This study introduces several significant features for consideration: (1) the finding of the consistent ratio of epinephrine to norepineph-
rime in myocardial tissues and the ratio of atrial to ventricular tissue concentration of catecholamines in nonanesthetized dogs; (2) the unchanged catecholamine content of the myocardial tissues after thiopental anesthesia; (3) the increase of atrial and ventricular norepinephrine content caused by ether and cyclopropane anesthesia; (4) the depletion of myocardial tissue epinephrine owing to ether, cyclopropane, and halothane anesthesia; (5) the relationship of tissue catecholamines to the plasma concentrations; and (6) the lack of myocardial catecholamine response in the anesthetized, reserpinized animals.

Cardiovascular Tissue Catecholamines in Control Dogs. The average norepinephrine concentration in atria was significantly higher (P < 0.001) than in the ventricles; the atrial-ventricular ratio being 1.8:1 for epinephrine and 2:1 for norepinephrine. The ratio of norepinephrine to epinephrine in the myocardial tissue was 10:1. Concentrations of norepinephrine in the aortic arch and in the pulmonary artery were almost as high as in the ventricular muscle.

Our results concerning the norepinephrine and epinephrine content in the control series are in general agreement with reports from other laboratories. In view of the uniformity of findings, these control values were used as a baseline for statistical comparison with the values obtained in the anesthetized group.

The Catecholamine Content after Thiopental. The values of the atrial and ventricular myocardial catecholamines were unchanged following thiopental anesthesia. This study does not substantiate the contention of others.
that thiopental causes liberation of norepinephrine from the arterial wall.\textsuperscript{4} It has also been shown that the barbiturates do not evoke a sympato-adrenal discharge.\textsuperscript{2, 3, 4}

**Increase of Norepinephrine Caused by Ether and Cyclopropane.** The storage content of the catecholamines in the myocardial tissue, especially the free or available portion of the stores, varies with the activity of the sympato-adrenal system.\textsuperscript{9, 12, 15, 16, 21-24} In addition to the adrenal glands, catecholamines are stored in postganglionic sympathetic neurones, especially the nerve endings and in scattered chromaffin cells in various tissues, e.g., myocardium, carotid body, paraganglia pelvic plexes and the peripheral vascular walls. The static content of the catecholamines in tissues depends upon the abundance of adrenergic nerve endings and chromaffin cells, but their dynamic variation depends upon the extent of activity of these neuro-humoral-secreting structures. Numerous reports have appeared in the literature regarding the increase of catecholamines due to stimulation of the cardiac sympathetic nerves.\textsuperscript{19, 22, 24} The increase of norepinephrine concentration in the myocardium during ether and cyclopropane anaesthesia is, therefore, presumably due to the increase in the activity of postganglionic sympathetic nerve endings. This concept is in keeping with the cardio-hemodynamic changes known to occur during these two kinds of anaesthesia.\textsuperscript{2, 3, 25, 26} The absence of an increase in myocardial catecholamines during halothane anaesthesia indicates a lack of increase in sympato-adrenal activity.

**Depletion of Epinephrine Owing to Ether, Cyclopropane and Halothane.** Ether, cyclopropane and halothane caused a depletion of the epinephrine in the cardiovascular tissue. Depletion was more marked in the myocardial tissues than in the walls of the large blood vessels. In contrast, the barbiturates do not produce this depletion effect (table 2).

The following possibilities are considered to explain the mechanism of myocardial epinephrine depletion: (1) Increased utilization of epinephrine to support the cardiovascular system during anesthesia; the rate of utilization or “breaking down” of epinephrine exceeds the rate of biosynthesis; (2) epinephrine may be exhausted because of its release from the chromaffin tissue or less likely, from the storage compartments at the nerve endings during anesthesia; (3) anesthetic agents may block methylation of norepinephrine to epinephrine; (4) epinephrine in the myocardial tissues may depend upon the adrenal gland for a supply of fresh epinephrine to be transported and deposited at the nerve endings; during ether, cyclopropane or halothane anesthesia, this supply may be interrupted, hence a reduced source of epinephrine for the cardiovascular tissues; (5) anesthetic agents may increase the permeability of the cellular membrane or of the storage compartments, therefore, facilitating the loss of epinephrine.

Insulin causes a comparable action; a total loss of epinephrine from the medulla without affecting the content of norepinephrine.\textsuperscript{27, 28} The depleting effect upon epinephrine, therefore, may not be an exclusive property of these anesthetics.

Circulating catecholamines may be drawn from the adrenal medulla and deposited in cardiovascular tissues. Epinephrine tissue content, therefore, may depend upon the circulating concentration of epinephrine. Depletion of epinephrine at the storage area may indicate the exhaustion of epinephrine mobilization from the adrenal medulla. This may explain the discrepancies in results reported on plasma epinephrine concentration during anesthesia.\textsuperscript{29, 30}

**Relationship to Plasma Catecholamines.** The experimental procedure permitted development of equilibrium between the plasma catecholamine concentration and the tissue stores. Therefore, it is reasonable to expect plasma catecholamine concentration\textsuperscript{29, 30} to parallel the changes in tissue content. The epinephrine depletion could be overlooked by analyzing only the plasma concentration, as is the situation with ether and cyclopropane anesthesia. Analysis of tissue catecholamines is related directly to the changes at the nerve endings and therefore may be more important than the plasma values.

The plasma and tissue norepinephrine values changed in the same direction, i.e., both increased during ether and cyclopropane anesthesia. It is also of interest that both plasma
and tissue norepinephrine were not increased following halothane anesthesia. The lack of increase of norepinephrine in both plasma and tissue stores seems to be one of the strongest evidences for the lack of the sympatho-adrenal stimulation during halothane anesthesia.

Absence of Catecholamine Response Following Reserpinization. The norepinephrine content in the cardiovascular tissues was not increased during ether and cyclopropane anesthesia in reserpined dogs. Storage mechanisms for norepinephrine are impaired by reserpine and, therefore, the anesthetic agents could cause no additional change. Reserpine depletes catecholamines, especially norepinephrine, from adrenal glands, sympathetic nerves, heart, and hypothalamus. The depletion of the norepinephrine from the sympathetic neurone fibers and nerve endings interferes with the transmission of the nerve impulse between the nerve endings and the effector organ. Therefore, stimulation of the accelerans nerve in a reserpined animal fails to cause cardio-acceleration.

The finding that in the reserpined dogs both ether and cyclopropane does not evoke an increase of norepinephrine in the adrenergic nerve endings in the atrial tissue is explained by the fact that the storage mechanism for norepinephrine is hindered by reserpine. In contrast, both ether and cyclopropane increased norepinephrine content in the nonreserpined preparation. The epinephrine content was not reduced to the same degree as the norepinephrine in the reserpined animals, both in the control state and during anesthesia. A probable explanation is that the storage mechanisms for epinephrine and norepinephrine are fundamentally different and hence are differently affected by reserpine. It has been reported that reserpine liberates only norepinephrine from the adrenal gland. The conservation of epinephrine in the cardiovascular tissues of reserpined animals may in part explain the apparently maintained integrity of cardiovascular function during anesthesia.

Summary

Catecholamines, epinephrine and norepinephrine, were determined in the right and left atria, right and left ventricles and the walls of the aorta and pulmonary artery in 61 dogs. Of the 45 nonreserpined dogs, 9 were controls and 9 each were anesthetized with thiopental, ether, cyclopropane and halothane. Of the 16 reserpined dogs, 4 were controls and 4 each were anesthetized with thiopental, ether and cyclopropane.

In nonreserpined, control animals, the catecholamine content in the atria was greater than in ventricular tissue; the atrial-ventricular ratio was approximately 1.8:1 for epinephrine and 2.1 for norepinephrine. The ratio of norepinephrine to epinephrine in atrial and ventricular tissues was 10:1.

In nonreserpined dogs, the catecholamine contents of cardiovascular tissues were not significantly changed following thiopental anesthesia, but norepinephrine content was significantly increased during ether and cyclopropane anesthesia in contrast to the significant depletion of epinephrine following ether, cyclopropane and halothane, in that order of magnitude.

In the reserpined group prior to anesthesia, 95 per cent of norepinephrine stores was depleted, with some reduction in epinephrine. Anesthetics were found to exert no effect upon the catecholamine contents of reserpined animals. The unaltered tissue epinephrine content of the reserpined dogs was in strong contrast to the depletion following ether and cyclopropane in nonreserpined dogs.

The mechanisms of these changes were discussed in reference to the hemodynamic effects of anesthetic agents.

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References


