Effects of Anesthesia on Norepinephrine Kinetics

Comparison of Propofol and Halothane Anesthesia in Dogs


Alteration of sympathetic function is a major determinant of the cardiovascular effects of anesthetic agents. Plasma norepinephrine (NE) concentrations are determined not only by the rate of NE release from sympathetic nerves but also by NE clearance rate. Therefore, NE concentration in plasma may be an inadequate index of sympathetic activity. We used an isotope dilution technique to investigate the effects of halothane and propofol anesthesia on NE kinetics. A relationship of NE kinetics to halothane dose was determined in six dogs. Halothane 1.0 MAC reduced plasma NE concentration by 35 ± 9% versus awake (P < 0.05). This was due to a reduction of 52 ± 5% in NE spillover (P < 0.05) accompanied by a reduction of 27 ± 5% in NE clearance (P < 0.005). The clearance changes were dose-dependent: reductions were 34 ± 4% at 1.5 MAC (P < 0.05 vs. 1.0 MAC) and 45 ± 5% at 2.0 MAC (P < 0.05 vs. 1.5 MAC). Six dogs were studied with a single halothane dose (1.0 MAC) and NE concentration, spillover, and clearance were found to be stable over a period of 5.5 h of anesthesia. Propofol (6 mg/kg followed by 0.8 mg·kg⁻¹·min⁻¹, n = 8) was equivalent to 2.0 MAC halothane in terms of spillover reduction (73 ± 5% vs. 72 ± 4%, P < 0.05), yet propofol had significantly less effect than did 2.0 MAC halothane on clearance (22 ± 4% reduction vs. 45 ± 5%, P < 0.01) and on systolic blood pressure (23 ± 5% reduction vs. 43 ± 3%, P < 0.01). Our data demonstrate that both halothane and propofol in anesthetic doses produce significant sympathetic inhibition. They also show that halothane and propofol differ in their relative potencies for spillover reduction and clearance reduction. Plasma NE concentrations alone are therefore inadequate for comparison of the sympathetic inhibitory effects of these two agents. (Key words: Anesthetics, intravenous; propofol. Anesthetics, volatile: halothane. Sympathetic nervous system, norepinephrine: clearance; spillover.)

The sympathetic nervous system is essential for the integration of autonomic function throughout the body. Cardiovascular function, and in particular arterial blood pressure, is determined in large part by the regulation and integration of sympathetic responses that modify heart rate, myocardial contractility, venous capacitance, and arterial resistance. The level of sympathetic activity during anesthesia is therefore of considerable importance; for example, the hypertensive effects of laryngoscopy and tracheal intubation are well recognized and are accompanied by increased sympathetic effector activity and plasma norepinephrine (NE) concentrations. Perioperative ischemic morbidity may be related to hemodynamic changes associated with increased sympathetic activity during surgery, and patients with heart failure who exhibit long-term sympathetic activation and high plasma NE concentrations are expected to have diminished regulatory control of arterial blood pressure during anesthesia.

It has been known for many years that halogenated anesthetics generally exert profound cardiovascular depressant effects and in addition "depress the sympathetic nervous system." Although halothane has been shown to decrease plasma catecholamine concentrations in animals, similar studies in humans show an increase in plasma NE concentrations during the induction of halothane anesthesia and a subsequent return to baseline levels after continued administration of halothane at a lower concentration. Propofol has been demonstrated to produce arterial hypotension in conjunction with decreases in cardiac output and systemic vascular resistance. However, the contribution of the effect of propofol on NE release to this hemodynamic depression has not been investigated and thus requires definition. Recent studies demonstrating marked reduction in muscle sympathetic nerve activity with propofol induction suggest that NE release should be investigated during propofol anesthesia.

Early studies by Brewster et al. and Price et al. suggested that the effects of volatile anesthetic agents on arterial blood pressure correlated with the changes in plasma catecholamine concentrations. Since then, the relatively insensitive fluorometric technique for catecholamine assay has been replaced by radioenzymatic and high-performance liquid chromatographic methods, leading to the extensive use of plasma catecholamine concentrations as a measure of sympathetic activity during anesthesia. In many of these studies, plasma NE concentrations have been assumed to be an appropriate measure of sympathetic activity. Although this assumption may be valid in certain circumstances, it is important to recognize that plasma NE concentration is determined by NE clearance from the circulation in addition to the rate of NE release into the circulation. Thus, the effect of anesthesia on plasma NE concentration depends on the relative effects...
of anesthesia on NE release and clearance. NE spillover can be defined as the total rate of entry of NE into the circulation, and that this occurs primarily from sympathetic nerve endings has been confirmed in humans by the finding that only 2% of plasma NE originates in the adrenal medulla.

The purpose of the current study, therefore, was to investigate the effect of an inhalational anesthetic (halothane) and an intravenous anesthetic (propofol) on both NE spillover or appearance rate in plasma and NE clearance in an experimental dog model.

**Materials and Methods**

Approval for the study was obtained from the Vanderbilt University Animal Care Committee. Fifteen healthy female mongrel dogs (29.4 ± 0.5 kg, mean ± standard error of the mean) were studied. Cannulas were implanted into the right femoral artery and vein during pentobarbital anesthesia (25–30 mg/kg, intravenously) at least 7 days before the study was performed. In order to allow observed changes in NE kinetics to be attributed to the anesthetic state, it was essential that the baseline values be determined in calm, resting conscious dogs. Therefore, all dogs were trained to lie quietly without restraint. Female dogs were chosen because they are more readily trained than male dogs. In four to five training sessions, each lasting about 1 h, each dog was familiarized with the experimental room and procedures. Relaxed posture, slow deep respirations, and spontaneous eye closure were used as indications of adequate training.

Studies were performed with halothane (n = 12) and with propofol (n = 8). On each occasion the dogs were studied first awake and then during anesthesia; each dog was its own control. Five dogs were studied with both agents; one week was allowed for recovery between studies.

**ISOPOTE DILUTION TECHNIQUE**

NE spillover and clearance rates were determined by isotope dilution. For each determination a separate 50-min infusion of H-NE was performed. H-NE of specific radioactivity 42.1 or 43.7 Ci/mmol (New England Nuclear) was diluted to 1 μCi/ml in 0.9% sodium chloride with 1 mg/ml ascorbic acid. Infusion was via the femoral vein; 15 μCi in the 1st min was followed by 0.6 μCi/min for 50 min. With this regimen, plasma H-NE concentrations reached steady state by 20 min (fig. 1). Arterial blood samples (5.0 ml) were taken just before infusion and 20, 30, 40, and 50 min after the start of the infusion and assayed for H-NE and endogenous NE. Blood was collected in cooled EGTA and glutathione (CAT-A-KIT blood collection tubes, Amersham) and centrifuged, and the plasma was stored at −20°C until assayed. Each sample was replaced with twice its volume of 0.9% saline. On each day of experimentation, samples of the H-NE infusion solution were collected, stored, and later assayed (as described for the blood samples) to allow determination of the actual rate of H-NE infusion on each day.

At steady state,

\[
\text{Rate of administration} = \text{rate of elimination}
\]

thus as

\[
\text{Rate of elimination} = \text{CL} \times C_{ss}
\]

\[
= \text{rate of administration}
\]

where

\[
\text{CL} = \text{clearance}
\]

and

\[
C_{ss} = \text{plasma concentration at steady state}
\]

**Fig. 1.** Plasma endogenous NE (pg/ml) (triangles) and H-NE (dpm/ml) (circles) concentrations (±SEM) in awake dogs and in dogs anesthetized with halothane (group A: 1.0, 1.5, and 2.0 MAC) and propofol.
NE clearance rate

\[
\text{NE clearance rate} = \frac{^{3}H\text{-NE infusion rate}}{\text{steady-state plasma } ^{3}H\text{-NE concentration}}
\]

But also

\[
\text{NE spillover rate} = \frac{\text{endogenous NE spillover rate}}{\text{endogenous plasma NE}}
\]

Thus, the pharmacokinetic parameters were derived from the following equations:

NE spillover rate

\[
\text{NE spillover rate} = \frac{^{3}H\text{-NE infusion rate (dpm/min)}}{\text{steady-state plasma } ^{3}H\text{-NE concentration (dpm/pg)}}
\]

NE clearance rate

\[
\text{NE clearance rate} = \frac{^{3}H\text{-NE infusion rate (dpm/min)}}{\text{steady-state plasma } ^{3}H\text{-NE concentration (dpm/ml)}}
\]

ASSAY FOR MEASUREMENT OF ENDogenous AND TRITIATED NE IN PLASMA

NE concentrations were measured by high-performance liquid chromatography with electrochemical detection (μBondapak 10 μm, 3.9 × 300-mm column; Bioanalytical System LC-4B electrochemical detector; and Waters 740 Data Module). 3,4-Dihydroxybenzylamine was used as an internal standard, and samples were extracted with alumina at pH 8.8. The limit of detection was 10.8 pg/ml. The intra- and interday coefficients of variation were 5.1 and 7.5%, respectively. The high-performance liquid chromatography effluent coinciding with the NE peak was collected and counted by liquid scintillation. This allowed determination of plasma 3H-NE concentration without interference from tritiated metabolites.

STATISTICAL ANALYSIS

Data were analyzed by repeated-measures analysis of variance (ANOVA) followed by Student’s paired or unpaired t tests as appropriate. P < 0.05 was accepted as the minimal level of significance.

Results

The awake values for cardiovascular and NE kinetic parameters determined for the three study groups are shown in Table 1. No significant differences were demonstrated between groups.

Figure 1 illustrates plasma concentrations of endogenous and 3H-NE in dogs anesthetized with propofol or increasing doses of halothane (group A). Repeated-measures ANOVA confirmed that endogenous NE concentrations were at steady state throughout each infusion pe-
TABLE 1. Comparison of the Baseline Cardiovascular and Norepinephrine Kinetic Parameters for Awake Dogs before Propofol or Halothane Anesthesia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Propofol (n = 5)</th>
<th>Halothane (Group A: n = 6)</th>
<th>Halothane (Group B: n = 6)</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE (pg/ml)</td>
<td>136.7 ± 18.0</td>
<td>156.3 ± 29.2</td>
<td>158.7 ± 42.4</td>
<td>NS</td>
</tr>
<tr>
<td>NE SO (ng/min)</td>
<td>286.0 ± 56.5</td>
<td>336.1 ± 67.3</td>
<td>284.8 ± 73.2</td>
<td>NS</td>
</tr>
<tr>
<td>NE CL (/min)</td>
<td>2.119 ± 0.567</td>
<td>2.216 ± 0.177</td>
<td>1.828 ± 0.151</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>155.9 ± 2.8</td>
<td>158.7 ± 9.5</td>
<td>145 ± 6.0</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>71.3 ± 3.2</td>
<td>70.7 ± 6.2</td>
<td>64 ± 3.4</td>
<td>NS</td>
</tr>
<tr>
<td>HR (beats per min)</td>
<td>84.8 ± 9.8</td>
<td>83.3 ± 6.1</td>
<td>73 ± 8.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

n = number of dogs. NE = norepinephrine concentration; NE SO = norepinephrine spillover; NE CL = norepinephrine clearance; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; NS = not significant.

Anesthesia divided different effects on the plasma concentrations of $^3$H-NE and endogenous NE (fig. 1). During anesthesia with either propofol or halothane, endogenous NE concentrations decreased markedly whereas, in contrast, $^3$H-NE concentrations increased. Because the rate of $^3$H-NE infusion was identical during each stage of the experiments, the increase in $^3$H-NE concentrations indicates reduced $^3$H-NE clearance.

Calculated NE clearance and NE spillover rates, together with NE concentrations, are illustrated in figure 2 for dogs anesthetized with halothane in groups A and B. For both groups all three parameters were less during anesthesia than in the awake state (ANOVA, P < 0.05 in each case). In group A, plasma endogenous NE concentration decreased by 35 ± 9% from 156.3 ± 29.2 pg/ml (mean ± standard error of the mean, n = 6) when the dogs were awake to 94.5 ± 19.4 pg/ml (P < 0.05) while they were anesthetized with 1.0 MAC halothane. When the concentration of halothane was increased to 1.5 MAC and then to 2.0 MAC, the reductions in NE concentrations were 38 ± 5% and 49 ± 5% from awake values and yielded mean plasma NE concentrations of 96.5 ± 22.0 pg/ml (P < 0.02 vs. awake) and 84.9 ± 22.2 pg/ml (P < 0.001 vs. awake), respectively. The NE concentrations during 1.5 and 2.0 MAC halothane were not significantly different from those determined during 1.0 MAC halothane anesthesia (not significant, by ANOVA).

Plasma NE concentrations reflect both the clearance and spillover of endogenous NE. NE spillover rates decreased during halothane anesthesia (fig. 2a), resulting in reductions of 52 ± 9, 59 ± 5, and 72 ± 4% at 1.0, 1.5, and 2.0 MAC, respectively, from the awake value of 336.1 ± 67.3 ng/min. The NE spillover rates were thus 149.7 ± 2.1 ng/min at 1.0 MAC (P < 0.05 vs. awake), 140.1

FIG. 2. The effect of halothane anesthesia on NE concentration (picograms per milliliter), $^3$H-NE clearance (filters per minute), and NE spillover (nanograms per minute) in dogs. Data are expressed as mean values ± SEM. A: Group A. Awake and during 1.0, 1.5, and 2.0 MAC halothane. B: Group B. Awake and during 1.0 MAC halothane anesthesia for 5 h.
± 34.9 ng/min at 1.5 MAC (P < 0.005 vs. awake), and 99.9 ± 28.1 ng/min (P < 0.005 vs. awake).

Halothane anesthesia also resulted in a dose-dependent decrease in $^3$H-NE clearance. NE clearance decreased from 2.216 ± 0.177 l/min when the dogs were awake to 1.617 ± 0.174 l/min (P < 0.005 vs. awake) at 1.0 MAC, to 1.467 ± 0.133 l/min (P < 0.05 vs. 1.0 MAC) at 1.5 MAC, and to 1.222 ± 0.191 l/min (P < 0.05 vs. 1.5 MAC) at 2.0 MAC. This represents reductions of 27 ± 5% at 1.0 MAC, 34 ± 4% at 1.5 MAC, and 45 ± 5% at 2.0 MAC, respectively.

Thus, halothane anesthesia resulted in a decrease in NE spillover, but because of the simultaneous decrease in NE clearance, the magnitude of the decrease in spillover was not fully reflected in the changes in endogenous plasma NE concentrations. This was especially true at 2.0 MAC halothane, at which a 72% decrease in spillover was accompanied by only a 49% decrease in NE concentration.

The time course of NE clearance, spillover, and concentration values during 5.5 h of 1.0 MAC halothane anesthesia is shown in figure 2b. The absolute values of these three parameters and the percentage changes from the awake values did not differ significantly from 1.0 MAC halothane in group A. After the initial reductions, there were no further changes in NE concentration, spillover, and clearance during 1.0 MAC halothane at any of the subsequent time points (not significant, by ANOVA).

The effect of intravenous propofol anesthesia on NE kinetics is shown in figure 3. Propofol anesthesia resulted in a significant decrease in mean plasma NE concentration, from 136.7 ± 18.0 pg/ml in awake dogs to 45.3 ± 8.2 pg/ml (P < 0.001) in the dogs while anesthetized, a 65 ± 7% reduction (fig. 3). NE spillover decreased from 286.0 ± 56.5 ng/min while awake to 70.9 ± 121.7 ng/min (P < 0.005) during propofol anesthesia, a change of 73 ± 5%. The NE clearance rate during propofol anesthesia was 1.612 ± 0.175 l/min (P < 0.01 vs. awake), representing a reduction of 22 ± 4% from the awake value of 2.119 ± 0.267 l/min.

Figures 4 and 5 demonstrate the cardiovascular effects of halothane (groups A and B) and propofol anesthesia, respectively.

A comparison of the effects of halothane and propofol anesthesia on NE kinetics (fig. 6) indicates that at the propofol infusion rate used in this study, propofol produced a decrease in NE spillover similar to that produced by the highest concentration of halothane (2.0 MAC) anesthesia, whereas the decrease in NE clearance produced by pro-
Propofol was similar to that produced by the lowest (1.0 MAC) concentration of halothane. In addition, for both the propofol group and halothane group A, systemic blood pressures correlated well with NE spillover rates (fig. 7), but propofol produced a smaller decrease in systemic pressure than did halothane for equivalent spillover changes (fig. 6).

**Discussion**

This study clearly demonstrates that both inhalational anesthesia with halothane and intravenous anesthesia with propofol produce important changes in sympathetic function, evident as marked decreases in NE concentration, spillover, and clearance. In addition, plasma NE concentrations were significantly decreased by both halothane and propofol anesthesia in dogs that exhibited low baseline resting NE concentrations when unrestrained and awake. Plasma NE concentrations at a given time are the result of a balance between the rate of NE entry (spillover) into and the rate of NE removal (clearance) from the circulation. The net effect of these anesthetic agents on plasma NE concentration is the result of alteration in NE clearance as well as in NE spillover, and the relative potency for spillover reduction versus clearance reduction appears to be different for the two agents. Therefore, it is clear that measurement of plasma NE concentration alone may not allow an accurate assessment of changes in sympathetic nervous system activity during anesthesia; factors affecting NE clearance, for example, include changes in cardiac output and regional perfusion and neuronal and extraneuronal uptake mechanisms. NE spillover is determined by multiple factors, such as sympathetic nerve activity, presynaptic modulation of NE release, and the reuptake processes that remove some of the released NE before it enters the circulation. Thus, there are many factors that interact to modulate plasma NE concentration.

The isotope dilution technique used in this study is based on the assumption of "steady state" endogenous and tracer $^{3}H$-NE levels at the time of sampling. Statistical
analysis of $^3$H-NE and NE concentrations during the infusion regimen demonstrated that this was the case. Isotope dilution also requires that the rate of isotope infusion be a trace dose (as in our study) to ensure absence of pharmacologic effect by the infusion and to ensure that release of $^3$H-NE previously taken up into sympathetic nerve endings does not become a significant determinant of plasma $^3$H-NE concentrations. The latter situation would cause underestimation of both clearance and spill-over of NE. As described in the Results section, the $^3$H-NE infusion rate in the current study produced steady-state $^3$H-NE concentrations that never exceeded 1.2% of total plasma NE. In addition, measurement of $^3$H-NE concentrations in plasma samples taken prior to commencement of each new tracer infusion consistently demonstrated only background levels of radioactivity, indicating that even with multiple infusions of $^3$H-NE (as occurred during halothane anesthesia), release of $^3$H-NE was not a significant factor. Also a problem in the assessment of NE kinetics by the method used in this study is choice of sampling site. Because endogenous NE is released at two sites in series (the lungs and the systemic organs) while the radiotracer is infused at a single site, the specific activity of $^3$H-NE differs throughout the circulation, so that NE spillover varies accordingly. Although no specific activity value truly represents a central plasma pool, regional distortion is greatest for peripheral venous samples, and therefore, arterial sampling (as we have performed) is preferable.

It is of particular interest that in our study both anesthetic agents (halothane and propofol) decreased not only NE spillover but also NE clearance. The latter effect opposed the former so that changes in plasma NE concentration underestimated the changes in NE spillover. This was especially true when higher concentrations of halothane were administered, since these were associated with the greatest reduction in NE clearance. Rorie et al., studying isolated canine saphenous vein strips, demonstrated not only that halothane inhibits stimulation-evoked NE release from sympathetic nerve endings, but also obtained indirect evidence that halothane may impair clearance of NE from the synaptic cleft. However, other reports have indicated that NE reuptake mechanisms in heart or nervous tissue are unaffected by halothane. On the other hand, considering that pulmonary extraction accounts for 45% of total plasma NE clearance in humans, it is noteworthy that halothane has been shown to decrease NE reuptake by the lung. Detailed studies of the effect of propofol on NE reuptake have not been performed.

Although NE spillover is a more sensitive index of sympathetic nervous system function than is plasma NE concentration, it is important to recognize that the amount of NE entering the plasma actually represents a balance between NE release into the synaptic cleft and NE reuptake from the synaptic cleft. Thus, in humans, NE spillover to plasma represents only about 20% of total body NE release. Therefore, a reduction in NE spillover by anesthesia represents either reduced NE release into the synaptic cleft or increased NE uptake. We consider the latter mechanism unlikely, first, because other authors have demonstrated reduced or unchanged NE reuptake with halothane, and second, because in the current study NE clearance was decreased with both halothane and propofol.

Our data show that NE spillover, clearance, and concentration were stable over 5.5 h of 1.0 MAC halothane anesthesia, confirming that the progressive reduction in NE clearance from 1.0 to 2.0 MAC was indeed a dose response rather than an effect of prolonged anesthesia. In addition, it suggests that the recovery in systolic and diastolic blood pressure that occurred with time (fig. 4) was not due to sympathetic activation. Cardiovascular recovery during prolonged halothane administration has been postulated to be due to increasing sympathetic activity because this recovery has been prevented by β blockade.

Because we have not quantified the anesthetic potency of our propofol dose we cannot compare the effect of propofol on NE kinetics with that of an equipotent dose of halothane. However, a meaningful comparison can be made between doses of the two anesthetics, which are equipotent in terms of their ability to reduce NE spillover. Figure 6 shows that by this criterion, 2.0 MAC halothane is equivalent to the propofol dose used in this study. It can also be seen that for an equivalent reduction in NE spillover, propofol had significantly less effect on NE clearance and on systolic blood pressure than did halothane. The relative preservation of NE clearance associated with propofol may reflect a depression of cardiac output or inhibition of the mechanisms of NE reuptake or metabolism that is less than that associated with halothane anesthesia.

In summary, this study has clearly demonstrated that both intravenous and inhalational anesthesia with propofol and halothane have important effects on NE kinetics; halothane and propofol inhibit NE spillover into the circulation in vivo, and since both anesthetics decrease NE clearance, the extent of the change in NE release is underestimated by measurement of plasma NE concentration alone. In addition, whereas 2.0 MAC halothane is equivalent to the propofol dose used in terms of decrease in NE spillover, propofol anesthesia had less effect on NE clearance and systolic blood pressure than did halothane anesthesia.

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References


