Absence of Biochemical Evidence for Renal and Hepatic Dysfunction after 8 Hours of 1.25 Minimum Alveolar Concentration Sevoflurane Anesthesia in Volunteers

Thomas J. Ebert, M.D., Ph.D.,* Edward J. Frink Jr., M.D.,† Evan D. Kharasch, M.D., Ph.D.‡

Background: Sevoflurane is degraded by carbon dioxide absorbents to a difluoro vinyl ether (compound A) that can cause renal and hepatic injury in rats. The present study applied sensitive markers of renal and hepatic function to determine the safety of prolonged (8 h), high concentration (3%) end-tidal sevoflurane anesthesia in human volunteers.

Methods: Thirteen healthy male volunteers provided informed consent to undergo 8 h of 1.25 minimum alveolar concentration sevoflurane anesthesia delivered with a fresh gas flow of 2 l/min. Glucose, protein, albumin, N-acetyl-β-D-glucosaminidase (NAG), and α- and π-glutathione-S-transferase (GST) levels were analyzed in urine collected at 24 h before and for 3 days after sevoflurane anesthesia. Daily blood samples were analyzed for creatinine, blood urea nitrogen (BUN), alanine aminotransferase, alkaline phosphatase, and bilirubin concentrations. Circuit compound A and plasma fluoride concentrations were measured.

Results: During anesthesia, average and maximum inspired compound A concentrations were 27 ± 7 and 34 ± 6 (mean ± SD) and median mean blood pressure, esophageal temperature, and end-tidal carbon dioxide levels were 63 mmHg, 36.8°C, and 32 mmHg, respectively. The average serum inorganic fluoride concentration 2 h after anesthesia was 66.2 ± 14.7 μM. Results of tests of hepatic function and renal function (BUN, creatinine concentration) were unchanged after anesthesia. Glucose, protein, albumin, and NAG excretion were not significantly increased after anesthesia. Urine concentrations of α-GST and π-GST were increased on day 1 after anesthesia and α-GST was increased on day 2 after anesthesia but returned to normal afterward.

Conclusions: Prolonged (8 h), high concentration (3%) sevoflurane anesthesia administered to volunteers in a fresh gas flow of 2 l/min does not result in clinically significant changes in biochemical markers of renal or hepatic dysfunction. (Key words: General anesthesia; volatile anesthetics; low flow; nephrotoxicity.)

SEVOFLURANE is a potent volatile anesthetic that undergoes metabolism to inorganic fluoride and degradation by carbon dioxide absorbents to compound A (fluoromethyl-2,2-difluoro-1-(trifluoromethyl) vinyl ether). Fluoride has been implicated in renal injury after methoxyflurane and enflurane anesthesia1-5 but has not been associated with human injury in several well-controlled clinical studies using sevoflurane anesthesia.3-5 Compound A administration to rats, at a threshold of approximately 100 parts per million (ppm), produces renal injury characterized by proximal tubular necrosis that results in increased urinary excretion of glucose, protein, and the proximal tubular enzymes N-acetyl-β-D-glucosaminidase (NAG) and α-glutathione-S-transferase (α-GST).6-7 Higher compound A concentrations produce increases in serum creatinine and blood urea nitrogen (BUN). Studies in volunteers and patients undergoing long periods of sevoflurane anesthesia administered in a low-flow (<2 l/min fresh gas flow)8-10 or closed circuit11 regimen (which enhances sevoflurane breakdown to compound A) have documented the absence of harmful effects based on unchanged standard clinical markers of renal function (serum BUN and creatinine). However, these studies have been criticized for not evaluating more sensitive, albeit experimental, markers of renal injury.12 Recently, Eger et al.13 evaluated these “sensitive” markers of renal injury in volunteers receiving 8 h of 1.25 minimum alveolar concentration (MAC) sevoflurane in a fresh gas flow of 2 l/min. They reported significant glucosuria and proteinuria and increased uri-
nary excretion of the proximal tubule cell enzyme, α-GST, and a distal tubule cell enzyme called π-glutathione-S-transferase (π-GST) in the postanesthesia urine samples of the volunteers. Based on these biochemical results, Eger et al.\(^5\) concluded that prolonged administration of high concentrations of sevoflurane lead to significant, but transient, glomerular and proximal and distal tubular injury.

Because most previous earlier human studies did not evaluate sensitive markers of renal function, this volunteer study has been unchallenged in interpretation and accuracy. Most recently, two studies recorded sensitive markers of renal and hepatic function from patients receiving low-flow (1 l/min fresh gas flow) sevoflurane or isoflurane anesthesia for surgical procedures lasting up to 9 h.\(^{13,14}\) and these have failed to identify any adverse effects from sevoflurane despite compound A concentrations that in some cases exceeded those achieved in the published 8-h volunteer study of Eger et al.\(^5\) Although there were clear methodologic differences between the patient and volunteer studies, the renal outcomes were opposite those that might have been predicted. The patient studies should have been more likely to show renal dysfunction because the use of a lower fresh gas flow resulted in some instances in which patients were exposed to higher compound A concentrations than volunteers were. Because of these inconsistent findings, we established a multicenter investigation (using blinded laboratory analyses) designed to duplicate the 8-h sevoflurane exposure study in volunteers (Eger et al.\(^5\)) that evaluated the potential for renal and hepatic dysfunction resulting from 1.25 MAC sevoflurane.

Materials and Methods

Participant Selection

These studies were approved by the Human Subjects Review Boards of the Medical College of Wisconsin and the University of Arizona Medical Center. Volunteers provided informed consent and were enrolled if they had normal results of a medical history and physical examination and if they weighed more than 80 kg. None were taking prescription medications or illicit drugs. The volunteers were instructed to fast for a minimum of 6 h and abstain from tobacco the morning of anesthesia.

Instrumentation and Administration of Anesthesia

On the morning of the study, a 20-gauge venous catheter was placed, a venous blood sample was taken, and saline was infused at 1.5 ml·kg\(^{-1}\)·h\(^{-1}\). Subjects were positioned supine on a well-cushioned table and standard noninvasive monitors (electrocardiograph, pulse oximetry, and automated oscillometric blood pressure) were attached to monitor heart rate, mean blood pressure (MAPB), oxygen saturation, and end-tidal carbon dioxide. Expired sevoflurane concentration and carbon dioxide were monitored using a calibrated infrared gas analyzer (Ohmeda RGM 5250; Madison, WI). Core body temperature was measured using an esophageal probe, and a target temperature of 37°C was achieved with an external warming device (Bair-Hugger; Augustine Medical, Eden Prairie, MN). Data were recorded and averaged over 5 min before initiation of anesthesia and at 10-min intervals during anesthesia.

Anesthesia was induced with propofol (2 mg/kg), and tracheal intubation was facilitated with rocuronium (100 mg). Mechanical ventilation was instituted to maintain end-tidal carbon dioxide levels at 28–32 mmHg. Sevoflurane was delivered to maintain an end-tidal concentration of 3% (1.25 MAC in this age group). An Ohmeda anesthetic machine with a circle absorber circuit was used to deliver sevoflurane in a fresh gas flow of 2 l/min (1:1, air:oxygen). An “artificial nose piece” with a dead space of 90 ml was placed for humidification between the endotracheal tube and the Y-connector of the breathing circuit. Fresh carbon dioxide absorbent (Baralyme; Chemetron, St. Louis, MO) was used to fill upper and lower absorbent canisters. Temperature measured 1–2 cm from the bottom absorbent canister was recorded at 10-min intervals during anesthetic administration. An MAPB <50 mmHg was treated with head-down tilt, a 250-ml fluid bolus, or both. At 2-h intervals, the volunteers’ limbs were massaged, and the head and extremities were repositioned.

Inspiratory and expiratory limb gas samples were taken from an airtight stopcock system positioned at the junction of the plastic inspiratory and expiratory hosing with the anesthesia machine. Samples were drawn into 5-ml syringes at 2, 4, 6, and 8 h of anesthesia and analyzed for compound A levels. Compound A samples from the Milwaukee, Wisconsin, site were injected into airtight 30-ml glass vials and mailed overnight to Seattle, Washington, for analysis within 36 h of collection. Compound A concentrations were determined by gas chromatography with flame ionization detection using the same validated assay as described previously.\(^{15}\) Strict validation criteria were used to ensure comparable results from both laboratories. Each laboratory obtained similar analyte retention times, peak widths, area
RENAL EFFECTS OF SEVOFLURANE

Table 1. Physiologic Measurements, Baralyne Temperature, and Compound A Concentrations during 8 h of 3% Sevoflurane Anesthesia

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>5 h</th>
<th>6 h</th>
<th>7 h</th>
<th>8 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>67 ± 11</td>
<td>68 ± 8</td>
<td>70 ± 8</td>
<td>73 ± 9</td>
<td>75 ± 10</td>
<td>76 ± 10</td>
<td>79 ± 11</td>
<td>79 ± 13</td>
<td>78 ± 13</td>
</tr>
<tr>
<td>Mean pressure (mmHg)</td>
<td>89 ± 7</td>
<td>67 ± 6</td>
<td>62 ± 10</td>
<td>64 ± 6</td>
<td>64 ± 5</td>
<td>64 ± 7</td>
<td>63 ± 5</td>
<td>63 ± 5</td>
<td>66 ± 5</td>
</tr>
<tr>
<td>Baralyne temperature (°C)</td>
<td>33.7 ± 6.9</td>
<td>39.9 ± 4.6</td>
<td>41.0 ± 4.3</td>
<td>41.3 ± 4.0</td>
<td>41.2 ± 3.7</td>
<td>41.3 ± 3.7</td>
<td>41.2 ± 3.6</td>
<td>41.3 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>Inspired compound A (ppm)</td>
<td>30.0 ± 5.5</td>
<td>31.8 ± 4.9</td>
<td>30.0 ± 5.4</td>
<td>28.4 ± 8.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expired compound A (ppm)</td>
<td>18.7 ± 5.0</td>
<td>22.8 ± 5.8</td>
<td>22.2 ± 2.4</td>
<td>20.7 ± 3.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD.

response ratios, and area reproducibilities (<3% coefficient of variation), and the absolute area of the internal standard for each patient sample was required to be within 10% of those measured for the calibration standards.

Venous blood was taken approximately 2 h after the anesthetic was discontinued for analysis of serum fluoride. Samples were centrifuged and the serum was pipetted and frozen at −70°C for later analysis by an ion-specific electrode (Orion Corp., Boston, MA). After 8 h of 1.25 MAC sevoflurane, the anesthetic was turned off, and the volunteer was allowed to breathe spontaneously, when appropriate criteria were met, tracheal extubation was done.

Blood and Urine Samples

Twenty-four hours before the experimental day and for 3 consecutive 24-h periods after anesthesia, venous blood and urine were collected. In addition, 5–7 days after anesthesia, 24-h urine and blood samples were obtained from 9 of the 13 volunteers. Standard laboratory tests were used by the respective hospital core laboratories to derive serum and urine glucose, protein, and creatinine concentrations (hexokinase, benzethonium chloride, and picric methods, respectively). Laboratory personnel used an auto analyzer system and were blinded to the identification of all samples. The interday coefficients of variation for low and high concentrations of urine protein were 4% and 1%, and for low and high concentrations of urinary glucose they were 2% and 1%. Urine concentrations of albumin were determined by nephelometry, and the interday coefficients of variation for low and high concentrations of albumin were 6% and 4%. There were no significant differences in urine analyte concentrations between the Tucson and Milwaukee laboratories. Aliquots of urine were sampled from containers of shaken 24-h urine collections. One aliquot was added to stabilization buffer (Biotrin International, Dublin, Ireland) and frozen for later analysis of GST with a commercially available (Biotrin International) enzyme immunoassay kit. The interday coefficients of variation were 7% for α-GST at 5 ng/ml and π-GST at 30 ng/ml. Urinary NAG activity (units/ml) was determined colorimetrically using a commercially available method (Boehringer-Mannheim, Mannheim, Germany). Twenty-four-hour NAG elimination was expressed as the amount per milligram of urine creatinine.

Statistical Analysis

Median values for MABP, heart rate, esophageal and baralyne temperature, end-tidal anesthetic, and carbon

Table 2. Laboratory Measurements of Hepatic Function

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Preanesthesia</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 5–7</th>
<th>Upper Limit of &quot;Normal&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/l)</td>
<td>26 (3–56)</td>
<td>26</td>
<td>28</td>
<td>30</td>
<td>41</td>
<td>60</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/l)</td>
<td>58 (39–79)</td>
<td>66</td>
<td>65</td>
<td>69</td>
<td>72</td>
<td>136</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.9 (0.6–1.4)</td>
<td>0.8</td>
<td>0.8</td>
<td>0.9</td>
<td>0.7</td>
<td>1</td>
</tr>
</tbody>
</table>

"Normal" = defined for healthy normotensive subjects not undergoing anesthesia; ALT = alanine aminotransferase.

Values are mean (range).

Anesthesiology, V 88, No 3, Mar 1998
Table 3. Laboratory Measurements of Renal Function

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Preanesthesia</th>
<th>Post Anesthesia</th>
<th>Upper Limit of &quot;Normal&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2</td>
<td>Day 3</td>
</tr>
<tr>
<td>Pcreat (mg/dl)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>(0.8–1.2)</td>
<td>(0.7–1.2)</td>
<td>(0.7–1.2)</td>
<td>(0.8–1.1)</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>15</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>(9–23)</td>
<td>(8–21)</td>
<td>(10–21)</td>
<td>(10–21)</td>
</tr>
<tr>
<td>prot/crea (mg/g)</td>
<td>67</td>
<td>83</td>
<td>96</td>
</tr>
<tr>
<td>NAG (IU/day)</td>
<td>2.5</td>
<td>3.0</td>
<td>4.0</td>
</tr>
<tr>
<td>(0.9–5.5)</td>
<td>(1.4–4.4)</td>
<td>(1.3–7.7)</td>
<td>(0.9–11.3)</td>
</tr>
<tr>
<td>G-GST (µg/day)</td>
<td>3.7</td>
<td>8.1*</td>
<td>5.2</td>
</tr>
<tr>
<td>(0–11.1)</td>
<td>(1–4.4)</td>
<td>(1–7.7)</td>
<td>(0–11.3)</td>
</tr>
<tr>
<td>α-GST (µg/day)</td>
<td>6.3</td>
<td>14.7*</td>
<td>44.8*</td>
</tr>
<tr>
<td>(0.3–30.2)</td>
<td>(5.1–48.4)</td>
<td>(2.7–101.1)</td>
<td>(1.8–128.7)</td>
</tr>
<tr>
<td>Albumin (mg/dl)</td>
<td>11.0</td>
<td>28.4</td>
<td>32.1</td>
</tr>
<tr>
<td>(1.5–39.2)</td>
<td>(5.1–48.4)</td>
<td>(2.7–101.1)</td>
<td>(1.8–128.7)</td>
</tr>
<tr>
<td>creat clear (ml/min)</td>
<td>144</td>
<td>161</td>
<td>133</td>
</tr>
<tr>
<td>(95–196)</td>
<td>(86–226)</td>
<td>(96–184)</td>
<td>(99–166)</td>
</tr>
</tbody>
</table>

Values are mean (range).

*Normal* = defined for healthy normotensive subjects not undergoing anesthesia; Pcreat = plasma creatinine; BUN = blood urea nitrogen; prot/crea = protein (mg/day)/creatinine (g/day); NAG = N-acetyl-L-α-glucosaminidase; G-GST = γ-glutathione-S-transferase; α-GST = α-glutathione-S-transferase; creat clear = creatinine clearance.

*P < 0.01 versus preanesthesia.

† The normal values for α-GST and G-GST have not been established in this laboratory. The assay manufacturer has indicated that the upper limits for α and G-GST concentrations, based upon 75 urine samples from normal volunteers, were 25 and 20 µg/l, respectively, which correspond to 40 and 32 µg/day.

Dioxide concentration were determined. Other data are reported as means ± SD. Data were compared for each daily measure of renal or hepatic function against the preanesthesia baseline using Student's paired t tests and a multiple comparison adjustment was applied (Bonferroni) so that P < 0.01 was considered significant for plasma and renal data.

Results

The 13 volunteers were, on average, aged 25 yr (range, 21–33 yr) and weighed 86 kg (range, 75–107 kg). Resting heart rate was 67 ± 11 beat/min, and resting MAPB was 89 ± 7 mmHg (mean ± SD; table 1). Median heart rate, MAPB, esophageal temperature, and end-tidal carbon dioxide during the 8-h period of anesthesia were 75 beats/min, 65 mmHg, 36.8°C, and 32 mmHg, respectively. Total fluid administration during the study period was 1,050 ml saline. Average serum inorganic fluoride concentration 2 h after anesthesia was 66.2 ± 14.7 µM. The average compound A concentration was 30 ± 4 ppm, and the mean peak inspired compound A concentration was 34 ± 6 ppm (table 1).

There were no differences in compound A concentrations analyzed in Tucson, Arizona compared with compound A concentrations from Milwaukee samples that were analyzed in Seattle.

Tests of Liver Function

Liver function was evaluated from plasma concentrations of alanine aminotransferase, total bilirubin, and alkaline phosphatase and are displayed in figure 1 and table 2. No significant changes in these variables from the preanesthesia baseline were observed, and no values exceeded the laboratory upper limit of normal.

Tests of Renal Function

Table 3 shows the accepted standard markers of renal function, BUN, and creatinine and creatinine clearance. There were no significant changes in these indices of renal function. "Sensitive" markers of injury are shown in figures 2–4 and in table 3. We expressed protein excretion as a ratio; i.e., urinary excretion of protein was corrected for 24-h creatinine excretion. This derivation is most appropriate when voluntary, unsupervised 24-h collections are requested. Thus, if there is inaccu-
RENAL EFFECTS OF SEVOFLURANE

Fig. 1. Plasma alanine aminotransferase concentrations in 13 volunteers measured before anesthesia and on the 4 days after anesthesia. No significant changes in ALT from the preanesthesia baseline were observed after 8 h of 1.25 minimum alveolar concentration of sevoflurane. The scale on the y axis was chosen to be identical to that of a previous publication.\(^5\)

Fig. 2. Urinary glucose concentrations in 13 volunteers measured before anesthesia and on the 4 days after anesthesia. There were no significant changes in average urinary glucose concentrations on any day after 8 h of 1.25 minimum alveolar concentration of sevoflurane. Two men had urinary glucose concentrations that exceeded the upper limit of normal on the first day after sevoflurane. The scale on the y axis was chosen to be identical to that of a previous publication.\(^5\)

Fig. 3. Urinary excretion of protein corrected for daily creatinine excretion in 13 volunteers measured before anesthesia and on the 4 days after anesthesia. There were no significant changes in average urinary protein concentrations on any day after anesthesia. One man had a urinary protein concentration that exceeded the laboratory upper limit of normal on the second day after anesthesia. The scale on the y axis was chosen to be identical to that of a previous publication.\(^5\)

There were no significant changes in average urinary glucose, albumin, or protein excretion on any day after anesthesia. Only 2 of 13 and 1 of 13 volunteers exceeded the upper limit of normal for urinary glucose and urinary protein and creatinine, respectively, and each occurrence was on a single day after anesthesia (figs. 2 and 3). The individual with an elevated urinary protein-creatinine concentration did not have any other renal marker that was increased above normal. One individual had a baseline (preanesthesia) urine albumin level of 39 mg/day that exceeded the laboratory normal limit. Four of 13 volunteers had 24-h urine albumin concentrations that exceeded the laboratory upper limit of normal on day 1 after anesthesia, and these increases persisted for 3 days, but all returned to baseline on days 5–7 after anesthesia (fig. 4). Two of these four persons also had an elevated urinary glucose level on day 1 after anesthesia. Mean values for enzyme markers of proximal tubule injury were significantly increased on days 1 and 2 after anesthesia for α-GST (fig. 5), but NAG concentrations were not significantly different from preanesthesia baseline on any day after anesthesia (table 2). Three of seven persons who had increases in α-GST after anesthesia had no other “sensitive marker” of renal function or injury that was above the upper limit of normal. The average concentration of the enzyme marker of distal tubule injury, π-GST, was significantly increased only on day 1 after anesthesia (table 2).

**Discussion**

This study, conducted in two separate research centers, shows that 1.25 MAC sevoflurane administration...
at the recommended fresh gas flow rate of 2 l/min for a period of 8 h to human volunteers is not associated with changes in biochemical markers that would be suggestive of clinically significant renal or hepatic dysfunction. We evaluated standard clinical markers of renal function and injury (plasma BUN and creatinine concentration) and collected consecutive 24-h urine specimens to evaluate more sensitive biomarkers (creatinine clearance, albumin, total protein, and glucose concentrations) and enzyme markers (NAG, α-GST, π-GST) suggestive of renal injury. Most of these markers remained within the normal range, with only a few exceptions when measured concentrations slightly exceeded the laboratory upper limit of normal. In addition, the plasma markers of hepatic function, including alanine aminotransferase, alkaline phosphatase, and total bilirubin, remained within the normal limits after 8 h of sevoflurane anesthesia.

These data from volunteers suggest no overt renal dysfunction after prolonged administration of sevoflurane and are in close agreement with two recent reports of renal outcomes in patients undergoing surgery who were anesthetized for as long as 9 h with sevoflurane or isoflurane delivered in a fresh gas flow of 1 l/min. Both patient studies also used sensitive markers of renal and hepatic function and no differences could be detected in these indices from patients randomized to receive sevoflurane or isoflurane.

**Experimental Design**

The methods used in this study were chosen to duplicate those described in a recent publication by Eger et al. that reported transient renal and hepatic dysfunction in volunteers after a prolonged sevoflurane anesthetic. The only methodologic differences between studies were the use of rocuronium in this study instead of vecuronium and the sole use of baralyme rather than the mixed used of either soda or baralyme by Eger et al. In the study, by design, we tried to maximize the generation of high inspired levels of compound A. This was achieved by using fresh baralyme, which leads to more compound A for a given sevoflurane exposure than does soda lime. In addition, volunteers with a large body mass were chosen because they eliminate more carbon dioxide per time compared with smaller persons. This results in higher temperatures of the carbon dioxide absorbent, thereby increasing the break-
down of sevoflurane to compound A.\textsuperscript{16,17} Finally, anesthetic adjuvants were not used, so that a high concentration of sevoflurane could be administered to attain and maintain an end-tidal concentration of 3%. This had a twofold effect. First, the more sevoflurane passing through the carbon dioxide absorbent, the higher the concentration of compound A in the inspired gases.\textsuperscript{16} Second, high concentrations of sevoflurane result in hypotension, and that can reduce blood flow to organs involved in the metabolism of sevoflurane and compound A. These conditions of high sevoflurane concentrations over 8 h, high carbon dioxide absorbent temperature, and fresh baralyme resulted in the exposure of unstimulated volunteers (i.e., no surgical stress) to the largest amount of compound A possible at a fresh gas flow rate of 2 l/min. Consistent with this goal, the measured compound A concentrations in the present research (average of 30 parts per million [ppm]) were as high as those reported with clinical use of sevoflurane in low (750 ml/min to 1 l/min) fresh gas flow (16 to 35 ppm)\textsuperscript{8-10,12} and closed circuit (13.6 to 30 ppm) conditions.\textsuperscript{11}

The Nephrotoxic Potential of Compound A

All potent volatile anesthetics degrade in the presence of the strong bases in carbon dioxide absorbents. In the case of sevoflurane, the degradation results in a vinyl halide called compound A that can be detected in the inspired and expired limb of the anesthetic breathing circuit. Studies that have administered high concentrations of compound A to rats have documented that the kidneys are the organ most susceptible to injury from compound A. The threshold for microscopic renal injury appears to be achieved when compound A is given over 3 h at 50–114 ppm.\textsuperscript{7,18} The primary injury to the kidneys is necrosis of the proximal tubule cells.\textsuperscript{5} The most sensitive markers of this injury are enzumuria (α-GST), glucosuria, and proteinuria.\textsuperscript{6,19} Greater exposures to compound A result in further renal injury, detected from increased plasma BUN and creatinine concentrations.\textsuperscript{6} Past clinical studies that have used low flow or closed circuit anesthesia with sevoflurane have resulted in compound A concentrations ranging from 13.6 to 35 ppm, and the average concentrations of compound A have not exceeded the threshold for toxicity defined for the rat model. These patient studies have demonstrated no adverse renal effects from sevoflurane and compound A exposure, but they have been criticized because sensitive markers of renal function (glucosuria, proteinuria, and enzumuria) were not evaluated.\textsuperscript{12} To address these concerns, one recent study used fresh baralyme and randomized patients to receive sevoflurane or isoflurane anesthesia delivered in a fresh gas flow of 1 l/min. Twenty-four-hour urine collections were evaluated for as long as 72 h after anesthesia and surgery. The duration of anesthesia ranged from 1.4 to 8.9 h and, in the sevoflurane group, compound A concentrations ranged from 10 to 67 ppm. Renal markers of dysfunction included 24-h glucose, protein, NAG, and α-GST excretion. There were no differences in the concentrations of these sensitive markers of renal function between patients anesthetized with sevoflurane and isoflurane.\textsuperscript{13} These data are consistent with the absence of laboratory evidence for significant functional abnormalities of the kidney in the present study, where volunteers were exposed to 8 h of 1.25 MAC sevoflurane and sensitive markers of renal integrity were used.

Noncongruous Results to a Previous 8-Hour Volunteer Study

This investigation was designed to duplicate the subject population, methods, and procedures described in a recent publication\textsuperscript{5} that reported biochemical evidence for significant but self-limited glomerular and proximal and distal tubule renal dysfunction and hepatic dysfunction in healthy volunteers after 8 h of 1.25 MAC sevoflurane delivered with a fresh gas flow rate of 2 l/min. This earlier study by Eger \textit{et al.} reported up to 4 g albumin and 27 g glucose in 24-h urine collections from volunteers who had undergone sevoflurane anesthesia. These marked changes were noted in the first few days after anesthesia, and all abnormal markers of injury returned to normal during the ensuing measurement periods. Although we duplicated the experimental design and methods of this earlier study, our outcomes differed substantially. Our peak urine albumin level was 131 mg/day and peak urine glucose was 1 g/day, which is 40 and 27 times, respectively, less than that reported by Eger \textit{et al.}\textsuperscript{5} One glaring difference between studies was the mean level of 30 ppm of compound A in the inspired gas in this study compared with 41 ppm in the volunteer study by Eger \textit{et al.} In addition, the average maximum compound A concentration was 34.5 ppm in this study and was 50 ppm in the previous one. An explanation for the divergence of compound A concentrations reported by Eger \textit{et al.} is not apparent. We matched all key factors that are important in the breakdown of sevoflurane to compound A, including fresh gas flow rate through calibrated flow meters; body mass of volunteers; sevoflur-
ane concentration; and type, manufacturer, and freshness of the carbon dioxide absorbent. We maintained identical body temperatures with active warming devices and we successfully duplicated the temperature of the baralyme measured from the lower canister of the carbon dioxide absorbent. We also limited fluids during the study period. Despite the successful duplication of the methods and procedures in the present study, Eger et al. delivered about 11 ppm higher inspired concentrations of compound A to volunteers. Whether the unusually high compound A concentrations delivered to volunteers in that study can account for the 40-fold differences in 24-h albumin excretion and the 27-fold differences in daily glucose excretion is unknown.

One additional difference in measured variables between this volunteer study and that by Eger et al. was the MABP recorded during the 8-h anesthetic administration. In the Eger et al. study, MABP averaged 56 mmHg compared with 62 mmHg in the present study. However, Eger et al. theorized that blood pressure was not an important contributor to the transient renal injury observed in their study. Given the recent clinical reports in which higher levels of compound A were inspired by patients who were not hypotensive without evidence of renal injury, and given the higher blood pressures in the present study, persistent low blood pressure should not be ruled out as a contributor or cofactor in the renal dysfunction reported by Eger et al.

There also may have been differences in the plasma inorganic fluoride levels between studies, but we only sampled blood for fluoride determination at one point in time. This sample was obtained 2 h after sevoflurane exposure, which is typically when plasma fluoride reaches its maximum. Our sampling was infrequent because of recent data that suggest that plasma inorganic fluoride from sevoflurane metabolism is not a mediator of renal injury. All but one of the fluoride concentrations in the present study exceeded the estimated threshold (50 μM/l) for fluoride nephrotoxicity defined for methoxyflurane. It appears that the intra renal metabolism of methoxyflurane may result in impaired urine concentrating ability, whereas the relative lack of renal metabolism of sevoflurane, and, therefore, lack of intrarenal fluoride, provides a greater safety margin for sevoflurane. Consistent with this theory, several rigorous investigations using tests of urine concentrating ability after 8-9.5 h of sevoflurane documented that renal concentrating ability was not compromised.

**Interpretation of “Sensitive” Markers of Renal Function**

Interpreting small but significant increases in sensitive markers of renal function is difficult because of the documented normal variation in these markers that results from physiologic stress. It is inappropriate to use the terms injury or toxicity when interpreting transient changes in renal markers of function unless histopathologic evidence of injury can be demonstrated. For example, transient proteinuria, up to 600 mg/day, has been reported during postural stress or after physical exertion. Most persons who have transient proteinuria during stress have normal histologic results on renal biopsy. Further, long-term follow-up evaluation, over a period of 41 yr, of healthy young persons with intermittent proteinuria indicates no significantly increased rates of morbidity or death when compared with a control group without measurable proteinuria. The experimental enzyme markers of renal tubule integrity also are not specific. For example, the urinary NAG excretion rate is increased by surgery, circadian rhythm, antibiotics, hypertensive episodes, prostatic hypertrophy, nonsteroidal anti-inflammatory drugs, and seminal fluid.

Based on this knowledge, in the present study, absence of overt renal or hepatic dysfunction from 8 h of 3% sevoflurane was concluded based on laboratory markers suggestive of dysfunction that remained close to, or less than, the “normal limit” described in laboratory manuals. These limits were derived from healthy persons who were not undergoing extended periods of anesthesia and hypotension in the recumbent position. In one recent study of patients receiving isoflurane for surgical procedures distant from the kidneys, so as not to compromise function, 24-h urine collections after surgery revealed average 24-h protein excretion rates of 200 mg/day with a range of 30 mg to 1 g of protein per day. Average glucose excretion was 1 g/day, and the range was from 20 mg/day to 20 g/day. The average and the maximal concentration of protein and glucose in the urine exceeded the laboratory normal limit. The recent study by Eger et al. indicates that some persons who were anesthetized for 8 h with a volatile anesthetic that was essentially not metabolized (desflurane) had increases in urine albumin, α-GST, and plasma alanine aminotransferase that exceeded the laboratory limit of normal. The authors’ interpretation of
these abnormal data was that renal or hepatic injury did not occur. Their subsequent implication that 8 h of sevoflurane anesthesia had significant adverse effects on renal and hepatic function was based on an empirical interpretation that more extensive deviations in these variables above the laboratory normal limit indicated "nephrotoxicity." In the present study, no volunteer had deviations above the normal limit for standard markers of renal function, including BUN and creatinine, whereas several volunteers had sensitive markers of renal and hepatic function that slightly exceeded the laboratory limit of normal. We have concluded that these small and transient increases in the biochemical markers do not represent significant changes in renal function or integrity because the concentrations were substantially less than those reported by Eger et al., and the markers were identical to the levels of urine glucose and protein measured in patients in the first 24 h after elective surgery with isoflurane anesthesia.13,14

The relevance to clinical anesthesia in surgical patients of this and previous volunteer studies is not certain. Surgical patients under anesthesia are not routinely maintained on 3% sevoflurane for 8 h under hypotensive, volume-restricted conditions as they were in these volunteer studies.

In summary, 8 h of sevoflurane anesthesia using a 3% end-tidal concentration (1.25 MAC) delivered with a fresh gas flow rate of 2 L/min does not result in clinically relevant renal or hepatic dysfunction based on sensitive enzyme and biomarkers of renal and hepatic function in humans. The obvious conflict in outcome and conclusions with an earlier study of similar design remains to be clarified.

The authors thank Richard Zager, M.D., Professor of Medicine, Division of Nephrology, University of Washington, Seattle, for his analysis of urine concentrations of N-acetyl-β-D-glucosaminidase, and Toni Uhrich (Milwaukee) and Susan Novak and Scott Morgan (Tucson) for their technical and analytical assistance.

References