Volatile Anesthetics Decrease Peristalsis in the Guinea Pig Ureter

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Background: The origin of renal dysfunction associated with anesthesia and surgery is complex and incompletely understood. The effects of the volatile anesthetic agents isoflurane, enflurane, and halothane on the renal pacemaker and ureteral peristalsis may play an important role.

Methods: Guinea pig ureter and pelvis were dissected and placed in a sample chamber that allowed immersing in a temperature-controlled bath with gas (O₂ with or without volatile agent) bubbled through the chamber continuously. The baseline frequency and amplitude of peristaltic contractions were measured on a polygraph recorder. The preparations were then exposed to up to 4 vol% volatile agent incrementally to generate a cumulative dose–response curve, and the subsequent frequency of peristaltic contractions was determined. The concentration of the volatile agent in solution was measured by gas chromatography.

Results: There was a significant dose-related decrease in the frequency of ureteral contractions for all three agents. A statistical model relating percentage baseline frequency to millimolar concentrations of volatile anesthetics showed that halothane produced a more pronounced decrease in frequency than did isoflurane or enflurane. However, the decrease was directly related to the MAC multiples and did not differ for the three agents.

Conclusions: Ureteral peristaltic contractions are decreased in a dose-dependent manner by enflurane, halothane and isoflurane. (Key words: Anesthetics, volatile; enflurane; halothane; isoflurane. Animals: Guinea pig. Kidney: pacemaker; peristalsis; ureter.)

Decreased urine output by patients during anesthesia and surgery is well known. It is commonly believed that urine output is directly dependent on intravascular volume and cardiac output, but the causes of decreased urine output in the peroperative period are incompletely understood. Urine production may be affected by a variety of factors, including changes in renal blood flow and changes in hormones (e.g., antidiuretic hormone, aldosterone, or angiotensin II). Decreased renal blood flow is temporally associated with decreased urine output under anesthesia, but the complete etiology of oliguria has not yet been determined. Although renal dysfunction in the perioperative period remains a common and significant cause of morbidity and mortality, assessing renal function from urine output is difficult and unreliable. Studies in the literature have not been able to correlate adequacy of urine output with postoperative renal function.

The argument that urine output relates to intravascular volume assumes that although anesthetic agents modify the determinants of renal function (such as renal blood flow), they do not alter renal function directly. We studied the direct, pharmacologic interactions of three commonly used volatile anesthetics on one aspect of renal function, the renal pacemaker and ureteral peristalsis. Located in the renal pelvis, the renal pacemaker regulates the peristalsis of the ureter. Urinary distension of the renal pelvis is thought to be one important modulator of renal function. In vitro studies have correlated urine flow with peristalsis of the ureter when no obstruction to flow is present. The interaction between volatile anesthetics and the renal pacemaker, as measured by spontaneous ureteral peristalsis, has not been studied to date and may contribute to perioperative oliguria. We tested the hypothesis that volatile anesthetic agents might attenuate ureteral peristalsis in a dose-dependent and drug-specific manner.

Materials and Methods

After approval by the Animal Care and Use Committee of the University of Chicago, guinea pigs were killed by CO₂ asphyxiation in a closed chamber. Their kidneys and attached ureters were removed immediately and
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Fig. 1. Time line (hours) of experimental method.

*Electrical Stimulation

maintained in oxygenated Krebs solution while the renal pelvis and ureter were dissected out. The ureteropelvic preparation was then tied with suture to a mechanical transducer (FT-03, Grass, Quincy, MA) and a preload of 200–300 mg was applied to each preparation. The transducer was connected to a polygraph recorder (3000 series, Gould, Cleveland, OH) via a universal amplifier (Gould). Four temperature-controlled (37°C) baths were used at one time. Each preparation was immersed in one of the baths, which contained 95% O2–5% CO2 Krebs solution. Composition of the Krebs solution was (millimolar) NaCl 119, KCl 4.7, NaHCO3 25, KH2PO4 1.2, CaCl2 2.5, MgCl2 1.5, and dextrose 11. Mixed gas (95% O2–5% CO2) with or without volatile anesthetic was continuously bubbled through the bath from an O2 cylinder in series with an agent-specific, temperature-controlled anesthesia vaporizer (for halothane, enflurane, or isoflurane) connected to a simple manifold to split the gas flow into the four baths. Each preparation was incubated for 30–60 min to allow dissection of the other preparations and stabilization of the baseline frequency. To verify the integrity of the autonomic neuroeffector junction, field stimulation was performed at the beginning and after each change in vaporizer setting by using electrical stimulation (10 s at 100–150 V) with a dual-pulse digital stimulator (S8800, Grass) as described by Maggi and Giuliani.7

After baseline measurements were obtained, the vaporizer was set at 1%. The measurements for this anesthetic concentration were obtained after a 20-min incubation (demonstrated as steady-state in a pilot study). Frequency measurements were obtained over 5 min after the incubation period. The Krebs solution was then changed and the vaporizer was set to a new level. A cumulative dose–response curve was generated with vaporizer settings of 1, 2, 3, and 4%. Other than a difference in achieved concentrations, results were similar with random order of vaporizer settings. At each level, the incubation period was 20 min, and the Krebs solution was changed afterward. Additional ureter preparations were used as time controls. Each ureter was exposed to only one anesthetic agent. The maximum viability of consistent ureteral peristalsis was about 5–6 h. The time course of the experiment is summarized in figure 1.

The bath concentration of anesthetic was measured because the baths were open to the atmosphere, and steady-state concentrations are dependent on the amount of bubbling, mixing, and tissue uptake. In a pilot study, bath concentrations attained a steady state after 20 min. By inference, tissue levels must also be constant at that level. The amount of bubbling in each bath was in part dependent on the number of baths being used but was constant over the course of each day.

Because no attempt was made to standardize the bubbling between baths, the gas chromatograph provided the only reliable measure of anesthetic concentration in the bath.8,9 The gas chromatograph contains a 1.8-m stainless steel, preconditioned coil column with 3% OV 17 packing (Alltech, Deerfield, IL). At all points where the contraction variables were measured, anesthetic concentration was measured simultaneously by placing 1-μl injections of the sample into the gas chromatograph. Aliquots of the bath solution close to the ureter preparation were obtained with a tuberculin microsyringe at the time of frequency measurement.
(steady state). Concentration of anesthetic was determined by comparison of the peak on the chromatograph with a known peak from a standard solution. The peak was calculated from the area under the curve or the peak height, if the waves were about the same width.

**Statistical Analysis**

A total of 38 preparations were obtained: 10 in the enflurane group, 16 in the halothane group, and 12 in the isoflurane group. In each group, two preparations were discarded because they were damaged during dissection, showed irregular contractions, or had poor contractility. Two additional halothane preparations were discarded for technical reasons. After these exclusions, 30 ureter preparations were included in the analysis: 8 in the enflurane group, 12 in the halothane group, and 10 in the isoflurane group. The total number of observations of frequency of contraction and concentration on these preparations was 144. Observations were excluded from the analysis if there was evidence of inadequate or inappropriate bubbling, i.e., if the measured concentration decreased with increasing vaporizer settings. Two observations of frequency and concentration were discarded for each anesthetic agent, leaving a total of 138 observations: 35 in the enflurane group, 55 in the halothane group, and 48 in the isoflurane group.

Each measurement of anesthetic concentration in millimoles was determined as the average of three independent measurements on each sample. The frequency of contraction, measured directly from the polygraph, was averaged over 5 min once steady state was achieved. Measured concentration was also converted to percent MAC (see Appendix) for comparison across anesthetic agents. Frequency of contraction was converted to percent of baseline to control for heterogeneity of baseline frequencies across preparations.

The relation between frequency of contraction and anesthetic concentration was examined with the use of nested linear regression models. The most liberal model allows a separate slope and intercept for each preparation, similar to the use of individual regressions. The next model allows separate intercepts, but assumes a common slope. The most parsimonious model assumes a common slope and intercept for all preparations. F tests were used to determine whether it was appropriate to move from more liberal to more parsimonious models. The more parsimonious model allows greater precision in estimating the dose–response relation and greater power in comparisons across anesthetic agents than do individual regressions. This series of models was examined separately for each anesthetic agent to allow for differences in variability of contraction frequencies across agents. Adjusted $R^2$ was used to account for multiple predictors in the linear regression models.

**Results**

Although incremental vaporizer settings were used, the distribution of dose concentrations for each anesthetic agent was nearly continuous and is shown in figure 2. Maximum concentrations (millimolar) were 1.11 for enflurane, 0.99 for halothane, and 1.14 for isoflurane. The baseline frequencies of the preparations (contractions per minute) were 4.4–6.0 (5.5 ± 0.5) (mean ± standard deviation) in the enflurane group, 4.8–6.6 (5.7 ± 0.5) in the halothane group, and 4.8–6.2 (5.5 ± 0.5) in the isoflurane group.

Anesthetic agents were compared on a molar concentration basis. Table 1 shows the results of F tests to determine whether a common slope and intercept could be used for all preparations, within each anesthetic agent. For all three anesthetics, it was possible that the preparations within each anesthetic had a common slope. For enflurane, each preparation had a distinct intercept ($P = 0.01$). For halothane and isoflurane, assuming a common intercept across preparations was reasonable. The final linear regression models, shown in figure 3, were:

- Halothane: % control = 83.0 − 104.1 mM $R^2 = 0.69$
- Isoflurane: % control = 91.1 − 76.5 mM $R^2 = 0.77$
- Enflurane: % control = intercept(i) − 63.7 mM $R^2 = 0.70$

![Fig. 2. Distribution of achieved concentrations. All preparations had a zero concentration point.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931299/)
Table 1. Statistical Models of Ureteral Peristalsis

<table>
<thead>
<tr>
<th>Test</th>
<th>Drug</th>
<th>F-test Values*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common slopes</td>
<td>Enflurane</td>
<td>0.86 (7, 19)</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>1.06 (11, 31)</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Isoflurane</td>
<td>0.81 (9, 28)</td>
<td>0.61</td>
</tr>
<tr>
<td>Common intercepts</td>
<td>Enflurane</td>
<td>5.04 (7, 26)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Halothane</td>
<td>1.31 (11, 42)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

* P-values shown with the degrees of freedom in parenthesis.

where intercept(i) in the enflurane equation denotes a preparation-specific intercept. For all regressions, the P value is < 0.01.

For enflurane, percent control response decreased 63.7% for every 1 mM increase in anesthetic concentration. For halothane, the decrease was 104.1%; for isoflurane, 76.5%. The slope of the halothane regression was significantly steeper than that of enflurane (P < 0.01) and of isoflurane (P = 0.017). The slopes of the regression lines for enflurane and isoflurane were not statistically significant (P = 0.26).

To relate frequency depression to anesthetic potency, we converted the measured bath concentration to MAC fraction, as described in the appendix. Maximum observed MAC ratios were 1.02 for enflurane, 2.00 for halothane, and 1.56 for isoflurane. The medians were 0.42 for enflurane, 0.66 for halothane and 0.64 for isoflurane. The adjusted R² values were the same as for millimolar models, because MAC ratios are linear transformations of millimolar concentration. Similarly, the results of F tests for determining whether to move to a more parsimonious model are the same as in table 1. The final linear regression models for MAC ratios were:

Halothane: % control = 83.0 - 51.7 MAC \( R^2 = 0.69 \)

Isoflurane: % control = 91.1 - 55.7 MAC \( R^2 = 0.77 \)

Enflurane: % control

\[ = \text{intercept}(i) - 69.7 \text{ MAC} \quad R^2 = 0.70 \]

For enflurane, percent control response decreased 69.7% for every 1 MAC increase in anesthetic concentration. For halothane, the decrease was 51.7%; for isoflurane, it was 55.7%. The difference between the regression slope for halothane and isoflurane was not statistically significant (P = 0.53), nor for enflurane and either halothane or isoflurane (P = 0.18 and P = 0.22, respectively). Because the slopes of the regression lines were not significantly different, the decreased frequency of ureteral peristalsis may be a function of anesthetic potency itself and not different for each agent.

Alternatively, as anesthetic depth increases, ureteral peristalsis slows with all the commonly used potent volatile agents.

Discussion

Decrease in the frequency of spontaneous ureteral peristaltic contractions was strongly dose-dependent and agent-specific with respect to concentration but

![Graphs showing the relationship between dose concentration and percent baseline frequency for Enflurane, Halothane, and Isoflurane.](http://anesthesiology.pubs.asahq.org/pdfaccess.ashx?url=/data/journals/jasa/931299/)

Fig. 3. Peristalsis frequency dose–response curves with linear regression line shown for halothane and isoflurane. The slopes were significantly different (P < 0.01). The slope of halothane was \(-104.10 \pm 9.54\) (m/s) and of isoflurane \(-76.46 \pm 6.11\) (m/s) (mean ± SE). For enflurane, each preparation had a specific intercept but the same slope. The line with the median intercept is shown, and the slope is \(-63.74 \pm 9.50\). The slope of enflurane was not significantly different from that of isoflurane. The slope of halothane was significantly different from the average of the enflurane and isoflurane slopes (P < 0.01).
not to MAC fraction. In other words, the depression in ureteral peristalsis seems to be related to anesthetic potency or depth. The effects of volatile agents on the renal pacemaker are not known because the physiologic and anatomic nature of ureteral peristalsis has not been completely characterized.

The existence of ureteral peristalsis and its initiation mechanism have been known for many years.\textsuperscript{11} Contractions in the ureter may be spontaneous or induced by chemical agents, physical distension of the ureter, limited sensory innervation, or applied electrical stimulation. Contractions occur at regular intervals, are generally peristaltic, are highly reproducible,\textsuperscript{2} and can occur only when the renal pelvis region is included in the sample preparation.\textsuperscript{12} The rhythmic contractions from the so-called renal pacemaker in the renal pelvis may lead directly to ureteral contraction or may be an integral multiple of the ureteral contractions that are modulated by the pyloureter.\textsuperscript{§} The spontaneous firing \textit{in vitro} of the renal pelvis pacemaker region and resultant ureteral peristalsis is tetrodotoxin-resistant,\textsuperscript{13} suggesting that the pacemaker resides in the pelvis and is not driven by extrarenal nerve inputs.\textsuperscript{14} That ureteral peristalsis itself is primarily a myogenic process being driven by a pacemaker source was demonstrated anatomically by Aragona \textit{et al.},\textsuperscript{15} who showed insufficient innervation from the autonomic nervous system between ureteral smooth muscle cells to initiate peristalsis. The ultrastructural construction of the pyloureter complex itself is part of the control mechanism; Constantinou\textsuperscript{12} and Zawalinski \textit{et al.}\textsuperscript{§} demonstrated that not all action potentials from the pacemaker are transmitted to the ureter, and that the ureteropelvic junction may function as a gating region for the symctium. The renal pelvis pacemaker and the ureter are therefore a functional symctium modulated but not driven by the autonomic nervous system.

Our \textit{in vitro} model did not include central autonomic nervous input, which is thought to be of little importance in ureteral function. The effects of acetylcholine on the renal pacemaker, first noted in 1928 by Gruber,\textsuperscript{2} are variable, usually small, and difficult to reproduce. The ureter complex may be stimulated \textit{in vitro} by nor- epinephrine via α adrenoceptors and is inhibited by β adrenoceptors, although this response varies among species.\textsuperscript{16,17} However, firm evidence is lacking for the existence of a functional, physiologically important adrenergic innervation of the pyloureter complex.\textsuperscript{18} Thus, many studies have used the isolated guinea pig model to investigate spontaneous ureteral peristalsis.

Although autonomic innervation has been shown to have little role, strong evidence exists for afferent sensory innervation, which may strongly modulate the renal pacemaker. The ureter has one of the highest concentrations of neuropeptides such as substance P or calcitonin gene–related peptide. These neuropeptides, contained in afferent sensory nerve fibers, are displaced by capsaicin.\textsuperscript{18,19} Numerous studies demonstrating the effects of calcitonin gene–related peptide,\textsuperscript{20} tachykinins,\textsuperscript{21,22} and bradykinin\textsuperscript{2} on ureteral peristalsis suggest the presence of specific receptors for these substances. Histamine, first noted in 1948 by Agar,\textsuperscript{11} is a profound stimulant of the symctium and has only recently begun to be characterized.\textsuperscript{23–25}*

We showed a strong effect of volatile agents on the frequency of ureteral peristaltic contractions, but in a preliminary study, we did not note any change in the amplitude of the contractions.\textsuperscript{26} Our results suggest that anesthetics affect the pacemaker itself or the proposed gating region, rather than the ureter smooth muscle cells. All three anesthetics produced dose-dependent decreases in peristalsis, although the variability of the endurane response was significantly greater than that of the other agents. This variability with endurane resulted in part from two aberrant preparations that we elected to keep in the analysis. At superanesthetic concentrations, the depressant effects of all of the agents taper off and possibly plateau. If this is the case, then simple linear regression may not adequately describe the effect. Unfortunately, we have insufficient data in the higher concentration range to allow a more complex model.

The greatest decrease in frequency occurred at less than 1.0 MAC, well within the therapeutic range. We infer, then, that urine transport under anesthesia is significantly reduced. Decrease in peristaltic frequency and urine transport may well contribute to oliguria, particularly in Trendelenburg position, in which the gravitational gradient is toward the kidney from the bladder. In our study, the demonstrated effect on the


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![Graph showing peristaltic frequency as a function of MAC.](image)

Fig. 4. Peristalsis frequency as a function of MAC. The linear regression lines for each agent are shown. The slope was $-51.65 \pm 4.73\%$/MAC for halothane, $-55.75 \pm 4.46\%$/MAC for isoflurane, and $-69.69 \pm 10.38\%$/MAC for enflurane. The enflurane line shown is for the median preparation-specific intercept. The difference between the slopes for halothane and isoflurane was not statistically significant, nor was the difference between the slopes for enflurane and halothane or between the slopes for enflurane and isoflurane statistically significant.

renal pacemaker–ureter syncytium is isolated from the rest of the kidney. In vivo, the ureter interactions and feedback controls on the other aspects of renal function may play a role in decreasing urine production. Other factors, such as changes in renal blood flow or hormones (antidiuretic hormone, aldosterone, or angiotensin II) may be involved in perioperative renal dysfunction.

The similarity between the renal pacemaker–ureter complex and the cardiac conduction system is apparent: both systems contain a dominant pacemaker region and a subsidiary conduction system that is modulated from internal and external sources. Although the effects of anesthetic agents on the cardiac system have been studied extensively and correlated with their clinical effects, to our knowledge, no such studies exist for the renal pacemaker. The electrophysiologic basis of anesthetic interactions on the heart affect the duration and amplitude of the action potential, phase 0 and 4 depolarization, and characteristic membrane potentials of the sinus node and conduction system. Studies have shown agent-specific and dose-dependent changes in all the measured variables of membrane potential. Furthermore, the site of action on the conduction system may be different for each anesthetic. By performing a similar series of experiments on the renal pacemaker system, it may be possible to characterize and identify selective advantages of specific anesthetics of use when renal function is compromised.

Research into ureteral peristalsis and the renal pacemaker has been sporadic to date, and the results do not give a complete picture of the initiation, modulation, and sequelae of this phenomenon. The strong depressive effects of volatile anesthetics on the renal pacemaker–ureter syncytium may prove to be an important determinant of urine transport in the perioperative period; the interactions of ureteral peristalsis with the other aspects of renal function have not been proven but may also play a role. Our findings suggest that direct pharmacologic effects on ureteral motility can occur with commonly used anesthetic agents. Previous studies of perioperative renal function focusing on renal blood flow and urine output have ignored these direct pharmacologic effects. With a better understanding of the renal pacemaker–ureter syncytium and its interactions with other anesthetic agents, it may be possible to tailor anesthesia and perioperative management to reduce the risk of renal dysfunction in the perioperative period.

The authors thank Professor G. Burnstock for invaluable direction and assistance in developing the model for the preparation.

Appendix

Because the potency of volatile agents (or MAC) varies among species, the statistical model was modified to account for the relative potency of the agents. Seifen et al. showed that for guinea pigs, unlike rodents, the MAC of halothane is 1.01 ± 0.03 vol%, of enflurane is 2.17 ± 0.04 vol%, and of isoflurane is 1.15 ± 0.05 vol%. Assuming that the corresponding solution concentration of volatile agent is the solution concentration that would be in equilibrium with the vapor phase and that volatile agents act as ideal gases, the temperature-appropriate Oswald water–gas solubility coefficients, $A$, for each agent could be used to calculate solution concentration at 1.0 MAC by the following relation:

Solution concentration (mM) = $0.393 \times %$ MAC (vol%)/$A$

Calculated with the Oswald coefficient of 0.80 for halothane, of 0.78 for enflurane, and of 0.62 for isoflurane, the solution concentrations (millimolar) at 1.0 MAC for guinea pigs are 0.496, 1.093, and 0.729, respectively, for the three anesthetics.

The model was then analyzed comparing percent control to percent MAC (for guinea pigs). These results are shown in figure 4. The maximum observed MAC ratios were 1.02 for enflurane, 2.00 for halothane, and 1.56 for isoflurane; the median MAC ratios were 0.42 for enflurane, 0.66 for halothane, and 0.64 for isoflurane. Because the MAC ratios were linear transformations of the dose concentrations, the use of percent baseline frequency in the MAC model is validated by the dose concentration model.
References


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